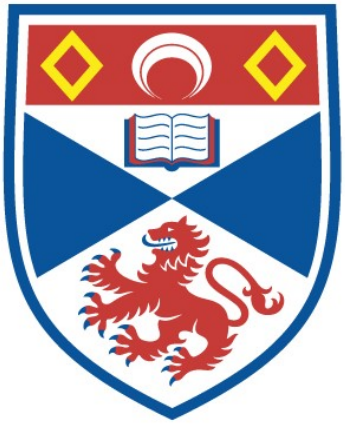
TIMING AND EPISODIC-LIKE MEMORY IN THE RUFOUS HUMMINGBIRD

Rachael E.S. Marshall

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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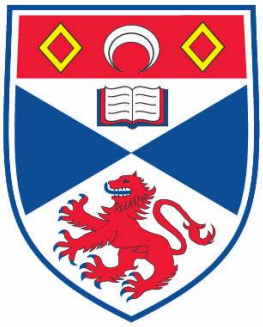

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at the

University of St Andrews

Date of Submission: 17.8.2012

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I, Rachael Marshall, hereby certify that this thesis, which is approximately 42000 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

I was admitted as a research student in October, 2008 and as a candidate for the degree of PhD in November, 2009; the higher study for which this is a record was carried out in the University of St Andrews between 2009 and 2012.

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Abstract

How animals remember past events has recently received a lot of attention, as researchers search for an animal model of episodic memory, the system used by humans to remember their pasts and imagine the future. It has now been repeatedly demonstrated that animals can remember what they did where and when, although how similar these memories might be to episodic memory remains controversial. Another broader point highlighted by this research is the variety of different ways an event's location can be specified in time, and how little we know of how animals in the real world organise their behaviour in time.

In this thesis I had two aims: to expand our understanding of the timing systems used by a free-living animal to organise its behaviour and, to look for novel ways of assessing the similarities and differences between animal and human memory. To this end, I investigated the timing abilities of free-living rufous hummingbirds *Selasphorus rufus,* in the Rocky Mountains of Alberta, Canada. In particular, I looked at the cues birds use to learn floral refill schedules, the types of temporal rules birds could learn, and the relationships between their memories for What, Where, and When. I also adapted a test used to study bird memory for use with human participants.

Together, the studies presented in this thesis suggest two potentially useful future avenues for research into human episodic memory: investigating whether animal memory is subject to similar distortions to human memory, and looking at human memory under similar situations to those used to test animals. This research also highlights the variety of temporal systems hummingbirds can use to guide their behaviour, and points to the study of timing as a potentially fruitful arena for investigating how an animal's cognitive abilities can be predicted by its environment.

Acknowledgements:

I would like to thank my supervisor Sue Healy and our collaborator Andrew Hurly for their guidance, patience and support during the completion of this thesis. I've learned so much from both of you, thank you for taking the time and trouble to turn me into a scientist. Thanks also to my second supervisor Gillian Brown, and to Thomas Morgan and Will Hoppit for their invaluable statistical help and advice. I would also like to thank Sally Rigg, Jenny Sturgeon and Nicola Wakefield for their help collecting some of the data which features in this thesis. This work was funded by the Natural Environment Research Council. Additional conference funding was provided by the Association for the Study of Animal Behaviour, and the University of St Andrews, Department of Psychology and Neuroscience.

Thanks to everyone who has worked at the Westcastle Valley over the course of this work: Jennifer Wiggins, Berenika Mioduszewska, Lauren Martin, Sarah Jelbert, Jennifer Iles, Thomas Fox and Christine Mishra, for help with the general business of looking after a field site, and making fieldwork unforgettable. Also my thanks to Ida Bailey and Cam Finlay for training me in hummingbird handling, and to Ida Bailey, James Ainge, Rosamund Langston, Barbara Dritschel, Michelle Arnold, Dave Shuker and Christelle Jozet-Alves for their invaluable practical and theoretical insights over the course of the last four years.

I would also like to thank the PhD students in the Wolfson who have shared the last four years with me: Felicity Muth, Guill McIvor, Emily Messer, David Pritchard, Maria Tellos Ramos, Zach Hall, Cris Cesar and Sally Street, and my friends and family beyond work: especially Alex, George, and Roy Marshall, and Eilis Cox and Alexandra Howe. Thank you all of you for putting up with me when work wasn't working, making me smile, and making the last four years into ones which I will remember happily. Particularly, thank you to my mum, Alison Marshall, for all your help, encouragement, patience and support, for this last four years, and the 22 before them.

Lastly, a special thank you goes to Kate Morgan and Nuri Abreu, who have shared this experience with me every step of the way: it wouldn't have been the same without you. Team Hummingbird forever!

Content Declaration

All experiments in this Thesis were designed by me in conjunction with Dr S.D. Healy and Dr T.A. Hurly, apart from Chapter 5, which was devised by Dr S.D.Healy and Dr T.A. Hurly.

All data were collected by me, with the following exceptions:

- Chapter 3: Data collected by Sally Rigg, under my supervision.
- Chapter 5: Data collected by Sue Healy and Jenny Sturgeon.
- Chapter 6: Data collected by me, and Sally Rigg and Nicola Wakefield under my supervision.

All analyses presented here are my own, and I was solely responsible for writing all of the chapters contained in this thesis as presented here, with revisions as recommended by my supervisors.

Dr T.A Hurly is a professor at the University of Lethbridge in Alberta. The University of Lethbridge own the field site where the majority of the data presented here were collected, and Dr Hurly has been a long-standing collaborator of Dr Healy in researching the hummingbird system used here.

Chapter 2 has appeared in print as "Marshall, R.E.S., Hurly, T.A. & Healy, S.D. (2012)."Do a flower's features help hummingbirds to learn its contents and refill rate?" Animal Behaviour **83**: 1163-1169." (see Appendix 1). This version contains an extra figure (Figure 3) and associated analysis, which was removed from the print version of this chapter for reasons of length. The experiment was designed by Rachael Marshall with additional input by Dr Healy and Dr Hurly. The data collection and analysis were my responsibility and the paper was written by me, with revisions as recommended by Dr S.D Healy and Dr T.A. Hurly.

Contents:

1: Introduction

Time is one of the most obvious factors influencing the environment that all animals experience. Many aspects of most environments change with time, and being able to predict and learn about temporal regularities and changes is a fundamental challenge for many animals. Broadly, in this thesis I am concerned with how animals learn about and predict these changes: what systems they use, how these temporal regularities are learned, how different timekeeping systems compare and interact with each other, and how animals compare with humans in how they remember the past and use this to guide their behaviour.

Recent research into how animals cope with changes in their environment over time has focussed mostly on the last of these questions. Humans, when required to remember the past, or imagine the future (Atance and O'Neill, 2001), typically use their episodic memory (Tulving, 1972). Episodic memory is widely recognised as one of the two memory domains that make up declarative memory (memory that can be consciously recalled, along with semantic memory; Squire et al., 1993). Whilst semantic memory is a store of knowledge of facts about the world, episodic memories are memories for personal experiences and events from our past (Tulving, 1983). At its inception, episodic memory was defined as a system for storing information about the what, where and when of past events (Tulving, 1972). However, it is possible to remember the what, where and when of an event for which one has no personal memory. Therefore, current definitions of episodic memory focus on the experience of the individual rather than on the contents of the memory itself (Tulving, 1983, 2002) such that episodic memories are those which the individual has the sense of belonging to a personal past. This focus on experience, rather than content has led to the

supposition that episodic memory is dependent on "complex" capacities including a sense of self, and an awareness of the self as a being with a past and a future (autoneoetic consciousness) and, therefore, that episodic memory is uniquely human (Tulving, 1983, 2002). However, this very focus on the experience of remembering makes the examination of possible episodic memory capacity in non-human animals practically impossible, as we do not yet have a plausible framework with which to infer mental states without language (Shettleworth, 2007).

Despite this, the question of whether or not animals may remember the past in the same way as humans has been particularly popular in recent years. This is partially because episodic memory has been identified as a form of "higher" cognition, but also is important for practical reasons: Episodic memory is attacked by Alzheimer's disease, which is a growing global problem and thus finding an appropriate animal model has particular importance for the development of drugs and treatments. Whether or not animals have similar memories of the past to humans also has animal welfare implications (Lea, 2001, Mendl and Paul, 2008): current welfare directives are based upon the idea that stressful events are transitory, but if animals can remember them as humans do this may mean we have to rethink some of our criteria regarding what is and is not acceptable to expose animals to.

This interest in animal models of episodic memory, coupled with the difficulty of testing how memory is experienced, has meant the extensive research into animal models has been based on a definition in which the aim has been to capture the functional, but not necessarily the phenomenological aspects of episodic remembering. Thus, the definition for animal episodic memory is based on Tulving's 1972 definition and requires animals to remember the what, where, and when of a specific past event,

and to be able to use this to guide behaviour (Clayton et al., 2003a). This phenomenon is now described as 'episodic-like' memory.

In the first test of episodic-like memory, Clayton and Dickenson (1998) investigated what Western Scrub Jays *Aphelocoma californica* could remember of their food caches. Birds were taught that a preferred food (waxworms) would be inedible after a long delay (124 hours) but would still be edible after a short delay (four hours), whereas a less preferred food (peanuts) would not degrade across the course of the 124 hours. Birds were allowed to store these waxworms and peanuts over two storage events 120 hours apart and then, four hours after the second storage event, to recover their caches. Birds preferentially searched for the preferred waxworms when they had been stored four hours ago, but after a long delay shifted their preference to the less preferred peanuts, demonstrating a memory for not only which foodstuffs had been stored where, but also how long ago they had been stored. Later work demonstrated that these birds can update their knowledge of food decay rates between storage and retrieval (Clayton et al., 2003c) and that they can direct their food storing in anticipation of future need (Raby et al., 2007).

This functional approach has proven extremely popular and has since been used as a model for testing a range of species, including rats, *Rattus norvegicus* (Babb and Crystal, 2005), magpies, *Pica pica* (Zinkivskay et al., 2009), chickadees, *Poecile atricapillus* (Feeney et al., 2009), meadow voles *Microtus pennsylvanicus* (Ferkin et al., 2008), mice (Dere et al., 2005, a system which has been used to begin to investigate the neurochemistry of episodic-like memory: e.g. Dere et al., 2008) and monkeys *Macaca mulatta* (although not successfully: Hampton et al., 2005). It now seems clear that a wide variety of animals are capable of remembering the what, where and when aspects

of past events and using them to guide their behaviour. Because of the differences between episodic and episodic-like memory, however, the extent to which this represents the working of a similar system to human memory remains contentious (Suddendorf and Busby, 2003b, Suddendorf and Busby, 2003a, Clayton et al., 2003b).

One way to determine how similar episodic and episodic-like memory are is to examine not only what information is stored in memory (the What, Where and When of episodic-like memory) but also how this information is stored. Human episodic memory is widely thought to be constructive: rather than storing memories as complete entities, episodic memories appear to exist as associations between components of an event, bound together at the time of encoding, and reconstructed from these associations at the time of recall, a reconstruction that may often be incomplete or incorrect (Conway and Pleydell-Pearce, 2000, Hassabis and Maguire, 2007, Schacter et al., 1998, Schacter and Addis, 2007). Indeed, the unreliability of memory is thought to be largely due to problems with the reconstruction of memories. For example, people remember words as having occurred in a list when they did not if the list contained a number of similar words (Read, 1996, Roediger and McDermott, 1995) and they confuse and conflate similar memories (described as blend errors: Nystrom and McClelland, 1992). These errors are thought to result from memories not being stored as whole entities (in the manner of a video recorder), but being remembered as associations between elements. This results in 'blend' errors when memories are similar, as associations effectively become "confused" between similar events.

Perhaps because the definition of episodic-like memory stresses that what, where and when must be remembered together, it is usually considered that an animal can only be deemed to have passed a test of episodic-like memory if it does remember

all three components together. However, the evidence supporting the constructive view of human memory comes from mistakes rather than successes. This means we currently have very little evidence regarding whether episodic-like memory resembles episodic memory in this regard. Pigeons' memories for symbols presented on screens over time periods of the order of seconds do not appear to show a binding together of what, where and when components, and memory for one component does not predict memory for another (Skov-Rackette et al., 2006). However, as the pigeons received extensive training in which they were only ever tested on either what, where, or when for each stimulus, directed forgetting may have interfered with their performance when the procedure was modified to allow testing of two memory components sequentially (Crystal, 2010). On the other hand, work with mice (DeVito and Eichenbaum, 2010) suggests that performance on an episodic-like task may share some structural similarity to human memory. Mice with hippocampal lesions were unable to remember all three aspects of a what, where, when task, whilst rats with prefrontal cortex lesions were selectively impaired in remembering where, which suggests some similarity of form between episodic and episodic-like memory, in implying separable neural substrates for at least some aspects of what, where, when memory. This picture is however further complicated by data which suggest there may be some redundancy in this system: rats with postrhinal lesions are impaired on a what, which task (Norman and Eacott, 2005), but perform normally on a what, where, which task (Eacott and Norman, 2004). Whether episodic-like memories are structured in a way that mirrors episodic memory, then, remains an unanswered question.

Comparing the structure of memory may be one way to address the differences between episodic and episodic-like memory but it is not the only strategy. An

alternative approach, rather than attempting to adjust human testing paradigms to use with animals as is usual, is to apply the tests we have developed for episodic-like memory in animals to humans (Holland and Smulders, 2011). For example, rather than hiding peanuts and waxworms, Holland and Smulders allowed their participants to hide coins of different values (20p and 2p) on two different days, and later asked them to remember which coin type had been hidden where on which day. The participants may have used an episodic strategy to solve the task as their performance was explained by their ability to remember other aspects of the hiding events. It is true that, if we find that humans use episodic strategies to solve episodic-like tasks, this would not necessarily imply that animals also do. However, by investigating a range of different species in a variety of different circumstances, we should be able to gain some insight into the similarities and differences between humans and other animals in their memories for past events.

Whilst adjusting animal paradigms for use with humans may help us to determine how similar episodic and episodic-like memory are, the situation is complicated by the number of different testing paradigms which have been developed in recent years, and the debate regarding which if these are more or less appropriate for investigating animal memories for past events. In particular, a long-running debate in episodic-like memory concerns the form that the "When" of "What", "Where", "When" memories ought to take. In the first study of this ability, Clayton and Dickinson (1998) specified the time of an event with regard to how long ago it occurred. Whilst many animals have since been found to be able to remember the time of a past event when specified in the same way (Babb and Crystal, 2005, Feeney et al., 2009, Ferkin et al., 2008, Zinkivskay et al., 2009), early studies with rats (Bird et al. 2003) and monkeys

(Hampton et al 2005) failed to demonstrate an encoding of the when of an event under this system. This, coupled with debates as to whether how long ago may be encoded by other systems, such as an interval timing system (Crystal, 2009, Raby and Clayton, 2009), the strength of a decaying memory trace or relative familiarity (Eacott and Easton, 2007, Roberts, 2002) or counting days passing (Roberts, 2002), has led to increased interest in studies which specify the time of an event in other ways. These include specifying the time of an event by its place in a sequence, for example, by giving rats single exposures to sequences of odours and testing their memory for the order of two of the odours from the sequence (Fortin et al., 2002).

In selecting a time-form for the when of episodic-like memory studies, it seems sensible to base our choices on how humans specify the time of past events. Whilst there is some evidence that time in human memory can be specified by how long ago (Friedman, 1991), the majority of work on human episodic memory suggests that the time of a memory, like other aspects of episodic memory, is largely reconstructive (Friedman, 2004, Friedman, 1993). Rather than explicitly encoding the time of a memory when it occurs, or using memory processes such as decay as a proxy for elapsed time, people usually date their memories via inference from a memory's contents, as location, other people in attendance and a variety of other aspects of a memory can generally be used to fit it within known time patterns. This idea of human memory thus allowing an absolute time relative to a known time scheme to be assigned to an event has led some researchers to adapt time of day as a useful a form of "when" for time to take in episodic-like memory studies. For example, in Zhou and Crystal (2011) rats were allowed to forage in a radial arm maze for food, within which whether or not a preferred food would replenish was predicted by the combination of the time of

day at which they initially discovered the preferred food and the presence or absence of this preferred food in the central compartment at test. An alternative approach also based on human memory characteristics requires the specification of an event's location in time via the context in which it occurred (Eacott and Norman, 2004, Easton et al., 2009), generally the colour and texture of the test arena. In this paradigm, as in human memory, time is not explicitly important, but rather it must be determined by other aspects of the event to allow the task to be solved correctly.

One of the key points highlighted by this debate is that there are a large variety of different timing systems available to guide animals' behaviour. All of these timing systems are at least somewhat familiar to behavioural researchers. Interval timing has been studied in detail for many decades (Staddon, 1970) and there are numerous models for the processes that might underlie animals' ability to keep track of elapsed durations from seconds to minutes (Gallistel, 1990, Gibbon, 1977, Killeen and Fetterman, 1988). Circadian timing is also a well-developed field and multiple species are capable of circadian time-place learning including garden warblers, *Sylvia borin* (Biebach et al., 1989), pigeons *Columba livia* (Saksida and Wilkie, 1994), hamsters *Mesocricetus auratus* (Cain et al., 2004), and bees *Apis mellifera* (Pahl et al., 2007). Bees, *Apis mellifera* can be taught an alternation task (Couvillon et al., 2003) and rats show spontaneous alternation, a trait that has been used extensively in studies of memory (Dudchenko, 2004). We also know that animals can learn longer sequences than simple alternations, including rats (Kesner et al, 1984), pigeons *Columba livia* (Scarf and Colombo, 2010), and monkeys *Macaca mulatta* and *Macaca fascicularis* (Chenet al., 1997, Orlov et al., 2000). The learning of these sequences has been well characterised. For example, we know that items at the ends of sequences are generally well

remembered, whereas intermediate items are not, a widely recognised feature of free recall of serially presented items known as the serial position curve (e.g. Kesner et al, 1984), and that, where similar items are presented together in sequences, these sequences may be broken into corresponding "chunks" by animals, which facilitates learning (Terrace, 1987). We also know that there are multiple ways in which sequences can be encoded, and there is some suggestion that which of these ways an animal uses may be taxonomically dependent. When pigeons learn sequences, they are hypothesised to do so via a form of chaining: birds learn that B comes after A, and that C comes after B, but seem to not seem to assign sequence elements an ordinal position e.g. A=1st, B=2nd, C=3rd (Terrace,1987, although see Scarf and Columbo, 2010). In contrast, monkey's seem to learn sequences in the second of these ways, demonstrated by the fact that monkeys can correctly judge which item appeared first from non-adjacent pairs of items in a sequence, and are able to learn sequences comprised of old elements from different sequences more easily if these elements maintain the same ordinal position between lists (Chen et al. 1997, Orlov et al., 2000).

Whilst we therefore know a great deal about many of these timing systems, our understanding of these different ways that animals may monitor time is fragmented. These systems are generally studied in isolation, with the exception of time-place research in rats, where timing systems are sometimes put into conflict, and no clear picture has emerged of the circumstances favouring one system over another (Crystal, 2009). Most studies also use laboratory animals, under conditions of extensive training, involving typically hundreds or thousands of trials. All of this work enables us to determine what animals are able to do (e.g. timing an interval or learning a sequence), however, how animals keep track of time away from the laboratory is much less clear.

Studies of sequential learning in wild animals are rare and, to our knowledge, interval timing has never been studied in the wild. When animals in laboratories are trained to sequences or to time an interval, conditions are deliberately manipulated such that there is little other information to be processed. Whether such systems are relevant or useful to free-living animals, which typically have to be trained and tested over much shorter intervals, whilst engaged in a range of other activities (such as avoiding predators, finding food and courting mates) is an open question. We do not know if some systems tend to be used more than others, whether some circumstances favour one type of cue over another, whether different species have different tendencies to each other, whether information is integrated between these different systems, or what happens when these systems are put into conflict. Completely independently from understanding how animal cognition compares to that of humans, addressing these questions is important for understanding how animals deal with changes in the environment over time that occur in the real world.

Beyond helping us to investigate animals' timing abilities in their own right, a better understanding of how these different types of timing abilities can be used to solve real-world problems may help us to better understand the relationship between episodic and episodic-like memory. It is worth noting that, whilst episodic studies investigating how memories are located in time typically find that time is reconstructed and contextual in nature (Friedman, 1993), these studies generally differ in form considerably from those we use with animals: people are either asked about their memories for past events, or tested on their memory for lists, and time is generally incidental. On the other hand, the tests we use for animals typically require them to attend to time, and render one particular type of timing cue important for solving a task

successfully. Humans are capable of all the types of timing discussed here: we can time short intervals, with the characteristic scalar variance described by SET (Ratkin et al 1998), are able to learn sequences, and exhibit the serial position curves seen in other animals: e.g. Jahnke, 1963). Whether these different kinds of time information can, under the more ecological scenarios we use in animal research, be used to provide the time of an episodic memory is not clear. However, potentially by better understanding these timing systems with relation to the sorts of situations in which we test episodic-like memory in animals, and further comparative research, we may be able to gain a better picture of how these different timing systems relate to episodic-like and episodic memory, and how these two memory systems relate to each other.

In this thesis, I attempted to address some of these questions through a series of foraging experiments conducted using male free-living rufous hummingbirds *Selasphorus rufus*, in the Rocky Mountains of Alberta. Studying cognition in the wild has its drawbacks, such as the lack of control for animals' prior experience, or over their engagement in other activities. However, there are benefits, such as increased ecological validity and reduced concern over animal welfare. By studying birds' behaviour using experimentally provided supplementary food, there is no need to food deprive them as motivation for them to participate. Equally, because birds are free-living, they can choose to participate, or not, as they can forage on natural food sources. This means that animals are unlikely to be especially stressed by the experiment itself. Finally, and most importantly, the question of whether the timing systems we have investigated in laboratory situations are applicable to real world problems seems most appropriately addressed in the wild.

My study species, the rufous hummingbird, is a small (5-9cm long, weight 2-5g) nectarivore native to North America (Healy and Calder, 2006). It overwinters in Mexico, but has the most northerly breeding range of any species of hummingbird, stretching from northern California to Alaska: a migration that is one of the longest, relative to body size, for any bird. This work was conducted in the birds' breeding range, in the Canadian Rocky Mountains in Alberta, where male rufous hummingbirds arrive in mid-May and depart from in mid-to-late-July. During the breeding season, males are aggressively territorial, defending areas of open forest meadow roughly 500m in diameter, in which they display to attract females. Within these territories, males do not tolerate other males, who are chased away, and territorial males will often chase off other, larger species of bird. Males also harass females who try to feed on their territories. This territoriality is crucial in making this system suitable for studies of learning and memory, in that it allows repeated measures to be taken from the same individual. Being nectar feeders, birds derive most of their calorific requirements from floral nectar, although they will also hawk for small insects. During the breeding season, in the area where this work is conducted, males feed roughly every ten minutes, and, when free-feeding, tend to take around 80- 100 µl of sucrose per visit, although exact intervisit intervals and meal sizes vary.

Hummingbirds can readily be trained to feed from artificial flowers and feeders of a wide variety of appearances, a trait that is also useful in allowing them to be studied. Previous work by our group demonstrates that birds cannot smell or see sucrose in these artificial flowers without sampling them (Hurly 1996). In early May, artificial feeders containing sucrose solution are hung from wires between trees, 2-3 metres above the ground, along the Westcastle Valley. These feeders are maintained

throughout the season and provide an *ad libitum* supply of 14% sucrose (weight/weight), which becomes the main food supply of the territorial males. A bird's vigorous defence of his feeder enables us to train him to feed from artificial 'flowers', which can then be manipulated to test various aspects of the birds' cognitive abilities. At the beginning of the field season, we catch territorial males, using a cage placed around his feeder, and mark him on the chest using coloured ink. These marks last for the duration of the breeding season, allowing individual birds to be identified without recapture, and ensuring that our data come from the correct bird.

This system has been used for research on cognitive systems for the last 20 years, with a considerable degree of success. Rufous hummingbirds have excellent spatial memory, which they prioritise for relocating rewarding flowers above a flower's colour (Hurly and Healy, 2002), and they can learn to locate flowers according to various rules. For example, when flowers are spaced closely together, birds tend to use the positions of flowers relative to each other to relocate a reward, whereas when flowers are further apart, they tend to use the flower's absolute position (Healy and Hurly, 1998). Birds can also learn spatial positions very quickly and can return to a rewarded location after a single trial, even in the absence of a beacon (Flores-Abreu et al., 2012, Hurly and Healy, 1996). They will also avoid flowers they have just emptied (Healy and Hurly, 1995). Beyond whether or not a given flower is rewarded, birds can learn about the amount and concentration of sucrose a flower contains, and can associate this information with a flower colour. This associative learning has been used to study risk sensitivity (Hurly and Oseen, 1999) and context dependent decision-making (Bateson et al., 2002, Bateson et al., 2003, Morgan et al., 2012).

Most importantly for my research, there is also evidence that rufous hummingbirds remember how long ago they visited a particular flower (Henderson et al., 2006). Henderson et al. presented three hummingbirds with an array of eight artificial flowers, half of which refilled ten minutes after they were last visited and half after twenty minutes. Over ten days of training, birds learned to return flowers that refilled after twenty minutes significantly later than to flowers that refilled after ten minutes. They also appeared to match their return times to flowers refill times quite closely. As they can remember where and when they have fed, it would seem that hummingbirds may be useful as a model of episodic-like memory. As with other systems where animals have learned to track how long ago, it is not, however, clear how hummingbirds keep track of this information. In this thesis, I used this system to address the following questions:

(1) what information hummingbirds use to learn floral refill intervals, and whether the learning of these refill intervals resembles interval timing over shorter time scales;
(2) whether hummingbirds can learn other temporal rules to predict the location of a reward;
(3) whether birds' memories for what, where, and when are separable in a way that is consistent with the constructive view of human episodic memory.

I also investigated the birds' ability to learn refill rates compared to their learning of discrete refill intervals. This latter is, arguably, a less ecologically realistic scenario than is gradual refilling of flowers**.** In particular, I tested the hypothesis that, when faced with flowers that refilled gradually over time, birds would forage optimally by matching their revisit rates to floral refill rates. This concept of matching behaviour to

rate of reward is frequently found in operant conditions, where the effect is known as the matching law (reviewed in Williams et al, 1988). Whilst flowers accumulating nectar differ from the variable inter-reward intervals used to examine rate in laboratory situations, the biological problem being mimicked is very similar, and if birds forage optimally from gradually refilling flowers then we should expect similar results. Finally, I developed a human version of a floral refill task, to compare the strategies used by people to solve one of the problems I set for the hummingbirds.

Chapter 2: Colour does not potentiate learning in the rufous hummingbird

Introduction

Many animals can use an object's colour to categorise rewards (e.g pigeons, *Columba livia*: Logue, 1980; mantis shrimps, *Odontodactylus scyllarus*: Marshall et al., 1996; chicks, *Gallus gallus*: Vallortigara, 1996; squirrel monkeys, *Saimiri sciureus* and common marmosets, *Callithrix jacchus*: Laska and Metzker, 1998; and dogs, *Canis familiaris*: Elgier et al., 2009) including a range of nectarivores (e.g. bumblebees *Bombus terrestris*: Raine and Chittka, 2008; and a range of butterfly species: Kandori et al., 2009). The ability to make use of colour as a categorising variable by nectarivorous species may be a useful way of discriminating between resources in nature, as the floral resources they exploit can vary considerably with respect to colour.

Red has traditionally been thought of as an important colour in the feeding of nectarivorous hummingbirds, due to the predominance of red coloration in hummingbird-pollinated flowers (e.g. Grant, 1966). However, tests of colour preference in hummingbirds have been less compelling. Birds may sometimes prefer red (Collias and Collias, 1968, Meléndez-Ackerman et al., 1997) but often they do not (Bené, 1941, Grant, 1966, Lyerly et al., 1950, Wagner, 1946). They will also shift their preference from red quickly if another colour becomes associated with a more valuable reward (Meléndez-Ackerman et al., 1997). The red coloration of hummingbird-pollinated flowers may be a floral mechanism for decreasing visits by other nectar feeders as although hummingbirds can see in colour throughout the visible light spectrum and into the near ultraviolet (Goldsmith et al., 1981, Goldsmith, 1980), insect vision is generally poorer at the red end of the visible light spectrum (Altshuler, 2003, Briscoe and Chittka, 2001, Raven, 1972).

Not only are birds able to see colour, they are able to use it to identify rewards. Hummingbirds can form associations between various colours and the presence or absence of a reward (Goldsmith and Goldsmith, 1979, Hurly and Healy, 1996), as well as a reward's magnitude (Bateson et al., 2002, Bateson et al., 2003), and its variability (Hurly and Oseen, 1999). They also appear to be able to gain these colour associations through observation of conspecifics (Lara et al., 2009). But although hummingbirds can learn colour-reward associations, in most cases where colour and location provide the same information, hummingbirds appear to prefer to rely on spatial cues, both with regard to making a choice between options of equal value, where they will tend to pick options in a particular place rather than of a particular colour (Lyerly et al., 1950, Miller and Miller, 1971, Miller et al., 1985) and in terms of learning whether an object is or is not rewarded (Goldsmith and Goldsmith, 1979, Hurly and Healy, 1996, Miller et al., 1985).

Despite hummingbirds' preference for spatial over colour cues, however, colour does, in some circumstances, facilitate learning. For example, hummingbirds learn the location of a reward set amongst non-rewarding options more quickly when all options are different colours than when they are all identical (Healy and Hurly, 1998, Miller and Miller, 1971), potentially because variation in colour enhances discrimination of similar options. The purpose of the current investigation was to discover whether rufous hummingbirds, *Selasphorus rufus* might also use colour in a different fashion, this time as a common cue to distinguish between flowers of different reward value. In previous work, where space and colour have been investigated together, colour has either provided the same information provided by space or has served as an aide to increase individual flowers' discriminability. Here, we wished to look at whether birds paid

attention to colour when it provided information on top of that provided by location, by allowing flowers to be classified into different "types". These birds can learn the refill rates of individual flowers, demonstrating a form of episodic-like memory (Henderson et al., 2006; as can green-backed firecrown hummingbirds, *Sephanoides sephaniodes*; González-Gómez et al., 2011), but it is not clear whether colour plays a role in this acquisition. We also asked whether colour could enhance the learning of reward concentration.

In these experiments, birds were presented with arrays of four flowers. Each of these flowers had one of two possible refill rates or concentrations of sucrose solution. In the Refill Rate Treatment, for one group of birds each of the two refill rates of the flowers was associated with a floral colour, while for the other group each flower was a different colour. In the Concentration Treatment, individual birds' performance was compared when fed from flowers that were either colour-cued to concentration or individually distinctive. We predicted that birds would learn the refill rates and the contents of the flowers more rapidly when those were distinguishable by their colour label.

Methods

This study was conducted at the University of Lethbridge Westcastle Field Station, Alberta, Canada, at 1400m elevation in the Eastern Rocky Mountains (49˚35' N; 114 ˚41' W). The subjects were 12 territory-holding, free-living, adult male rufous hummingbirds, each defending an established territory around an artificial feeder containing 14% sucrose solution (weight by weight, as are all other sucrose concentrations in this experiment). At the beginning of the field season, birds were

trapped by a qualified hummingbird bander using a trap surrounding the feeder and marked on the breast with non-toxic, waterproof coloured ink so that they could be distinguished without recapture. Birds were also banded to allow individuals to be identified between years. Trials were run from 7:00 to 20:00 hours Mountain Standard Time in May and June 2009 and in July 2010. All work was approved by the University of St Andrews Ethical Committee, conducted according to the requirements of the Canadian Council on Animal Care and was carried out under permits from Alberta Sustainable Resource Development and Environment Canada.

Initial Training

All subjects were initially trained to feed from artificial flowers. A 'flower' consisted of a syringe tip forming the "nectar" well, containing up to 600µl of 25% sucrose, surrounded by a circle of card painted orange using acrylic paint, of roughly 6 cm diameter, all of which was mounted horizontally on a 60cm high wooden stake. Flowers were refilled manually by the experimenter between visits.

Concentration Treatment

This experiment was conducted in July 2010. The subjects in this treatment were five male territorial rufous hummingbirds. Each bird was presented with four artificial coloured flowers in a 1-metre square array. Two birds were initially presented with four flowers of different colours (dark green, dark blue, light blue, yellow, pink or purple), two containing 40µl of 20% sucrose, and two containing 40µl of 30% sucrose. The remaining three birds were presented with two flowers of one colour, which contained 40µl of 20% sucrose and two of another colour containing 40µl of 30%

sucrose (i.e. flower colour signalled sucrose concentration, the Cued condition, see Figure 1). The flowers were coloured using acrylic paint. The reward-colour association was varied between birds and the positions of flowers of each type were pseudo-randomly assigned so that for some birds like concentrations were adjacent and for some they were diagonally opposite. After each feeding bout, all flowers visited were refilled manually by the experimenter. As this took less than a minute, and typical return times for birds feeding from this kind of apparatus are in the region of 10 minutes, we considered that it was unlikely that this would have affected the birds' behaviour. We assumed birds were able to discriminate among the flower colours we provided, both because hummingbird colour vision is good across the visible light spectrum (Goldsmith and Goldsmith, 1979, Goldsmith et al., 1981) and because rufous hummingbirds have successfully discriminated similar flower colours in other unrelated experiments (Bacon et al., 2010, Bateson et al., 2002, Bateson et al., 2003, Healy and Hurly, 1998, Hurly and Oseen, 1999, Morgan et al., 2012). Birds in each treatment group were allowed to make 60 flower visits.

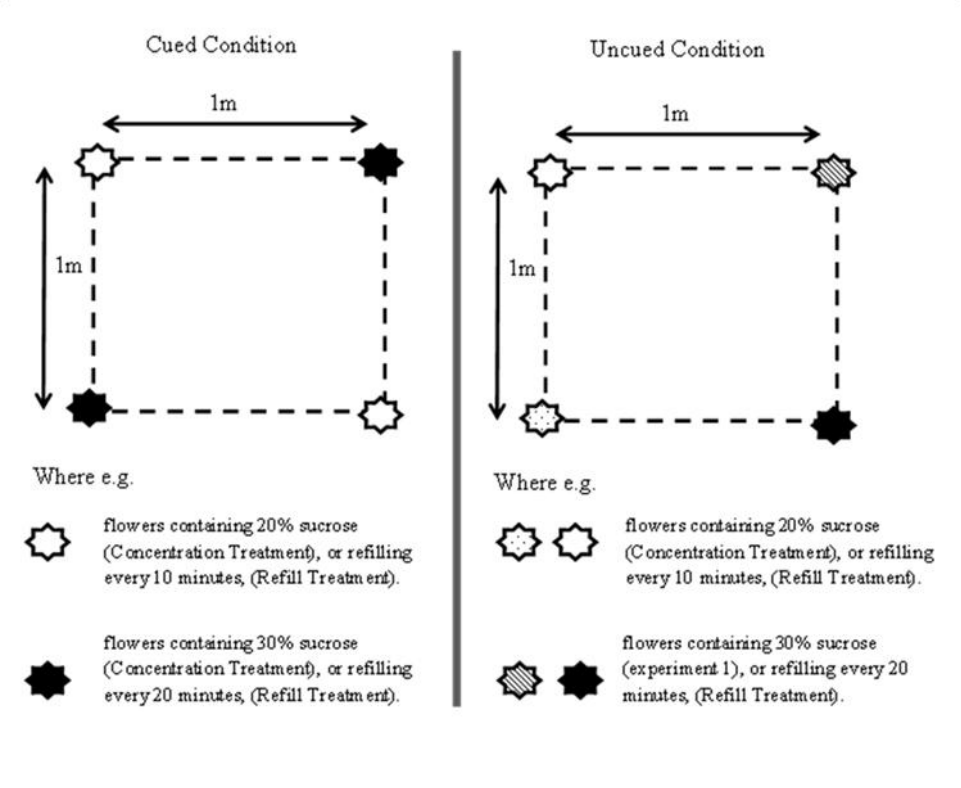

Figure 1. *Schematic of the experimental layout for the Concentration and the Refill Rate Treatments. For half of the treatments, the flowers of the same condition were adjacent to each other, for the other half they were diagonally opposite.*

All birds experienced both conditions. After completion of the first condition, the flowers were removed for at least an hour. This corresponds to 5-6 feeding bouts at hummingbirds' typical feeding rates. Each bird was then presented with the alternative condition (Cued condition followed by the Uncued or vice versa) using novel flower colours in a different location within their territory. Birds were again allowed to make 60 flower visits.

For each condition, we recorded all visits to the array made by the bird, the flowers he probed, the time of the visit and the order in which flowers were visited.

Refill Treatment

This experiment was conducted from May-July 2009. The subjects in this treatment were eight territorial male rufous hummingbirds, one of which was tested in the Concentration Treatment the following year. The birds were pseudo-randomly assigned to one of two experimental conditions, either the Cued or Uncued condition. The birds assigned to the Cued condition were presented with an array consisting of four flowers, two purple and two yellow. The birds in the Uncued condition were presented with four flowers, each a different colour (pink, purple, blue and yellow). Each flower colour for each bird was pseudorandomly assigned a refill time of either 10 or 20 minutes, such that two flowers for each bird were manually refilled 10 minutes after being emptied and two were refilled 20 minutes after being emptied. As for the Concentration Treatment, these flowers were positioned at the corners of a 1 metre square array. For half the birds in each condition adjacent flowers shared a refill schedule, while for the remaining subjects adjacent flowers were refilled on the alternate refill schedules (Figure 1).

When filled, each flower contained 25% sucrose. Due to large variation in birds' rates of feeding, the volume of sucrose in flowers was varied within and across days. It was increased in increments of 5µl if birds were consistently returning to the array before any flowers had refilled and decreased if birds were consistently returning less often than every ten minutes, to allow them opportunity to learn about the refill schedules of ten-minute flowers. Volume was varied simultaneously across flowers, so changes in volume should not have interfered with relative revisit rates between flowers, which was the response used in later analyses.

When a bird visited the array, the flowers it probed, the time of the visit and the order in which flowers were approached were recorded. A 20µl capillary tube was then used to measure and remove any remaining sucrose in all of the visited flowers. The visited flowers were then refilled either 10 or 20 minutes later. Birds were tested for a maximum of 10 hours per day.

The time taken to revisit a flower after the last reinforced visit (the post-reinforcement pause) was calculated for each bird visiting each flower across all training periods. If a bird did not visit the array for more than an hour during training, he was judged not to be participating in the experiment and the corresponding interval between visits was removed from subsequent analysis. This was to prevent long inter-visit intervals where we judged it unlikely that birds were attending to either refill rate from skewing the data. The data were then divided chronologically into groups of 50 post-reinforcement pauses (with the exception of the final training block for bird C2, which consisted of 24 visits and the final block for bird UC2, which consisted of 37 visits), and birds' post reinforcement pauses when visiting ten and twenty minute flowers compared using Mann-Whitney-U tests. A bird was taken to have learned the appropriate association when these return times were significantly different from each other. This block size of 50 visits was selected as a compromise between test reliability and power and for the grain of the scale over which it would allow comparisons. As a result the data were divided into up to seven blocks of 50, each containing 30.5 ± 3.5 (mean ± SD) visits to ten and 18.4 ± 2.7 visits to twenty-minute flowers respectively.

Testing ended either when birds had learned to discriminate between 10 and 20-minute flowers (as per the analysis detailed above) or when 25-30 hours of testing had elapsed. The 25-30 hour training cap corresponded to an average of 317 post-

reinforcement pauses, and ranged from 261-350. One bird discriminated between times within the first 50 post-reinforcement pauses and thus only contributed 79 post-reinforcement pauses.

Results

Concentration Treatment

In both conditions, birds selected the more concentrated option significantly more often than would be expected by chance (Cued condition: 0.65± 0.15 proportion of choices to the rewarded flower; one sample, one-tailed Wilcoxon signed rank test: $N = 5$, $Z = 1.75$, $P = 0.04$; Uncued condition: 0.63 ± 0.10 proportion of choices to the rewarded flower; one sample, one-tailed Wilcoxon signed rank test: $N = 5$, $Z = 2.06$, $P = 0.02$).

As can be seen from Figure 2, there appeared to be little to no effect of treatment on birds' choices. In order to investigate whether this was in fact the case, we developed a model using Bayesian methods to compare learning in the two groups, Bayesian methods being more suitable than traditional hypothesis testing for situations in which the data seem to support the null hypothesis (Gallistel, 2009). We investigated whether birds learned differently about floral concentrations in the presence of colour cues by constructing a generalised linear mixed model with a Bernoulli error structure and logit link function, modelling birds' choices of low or high flowers as a function of standardised visit number, trial type, and condition order. We controlled for the effects of individual birds on baseline preference, learning rate and difference between conditions. We used Markov Chain Monte Carlo Methods to develop the model in WinBUGS 1.4 (Lunn et al., 2000). The model was allowed to run for 10 000 iterations

with three independent chains for each parameter, with a suitable burn-in period to allow the three chains to converge. The chains were thinned to remove autocorrelations resulting in 1237 independent samples for each parameter. The distributions of these samples were then used to estimate parameters. This family of analysis is not based upon significance testing, but rather on parameter estimation, therefore we have provided median estimates and their 95% central credible intervals (CI), as is conventional for this type of analysis. A value of 0 indicates that a parameter had no effect on the data, whereas positive and negative values reflect positive and negative effects respectively.

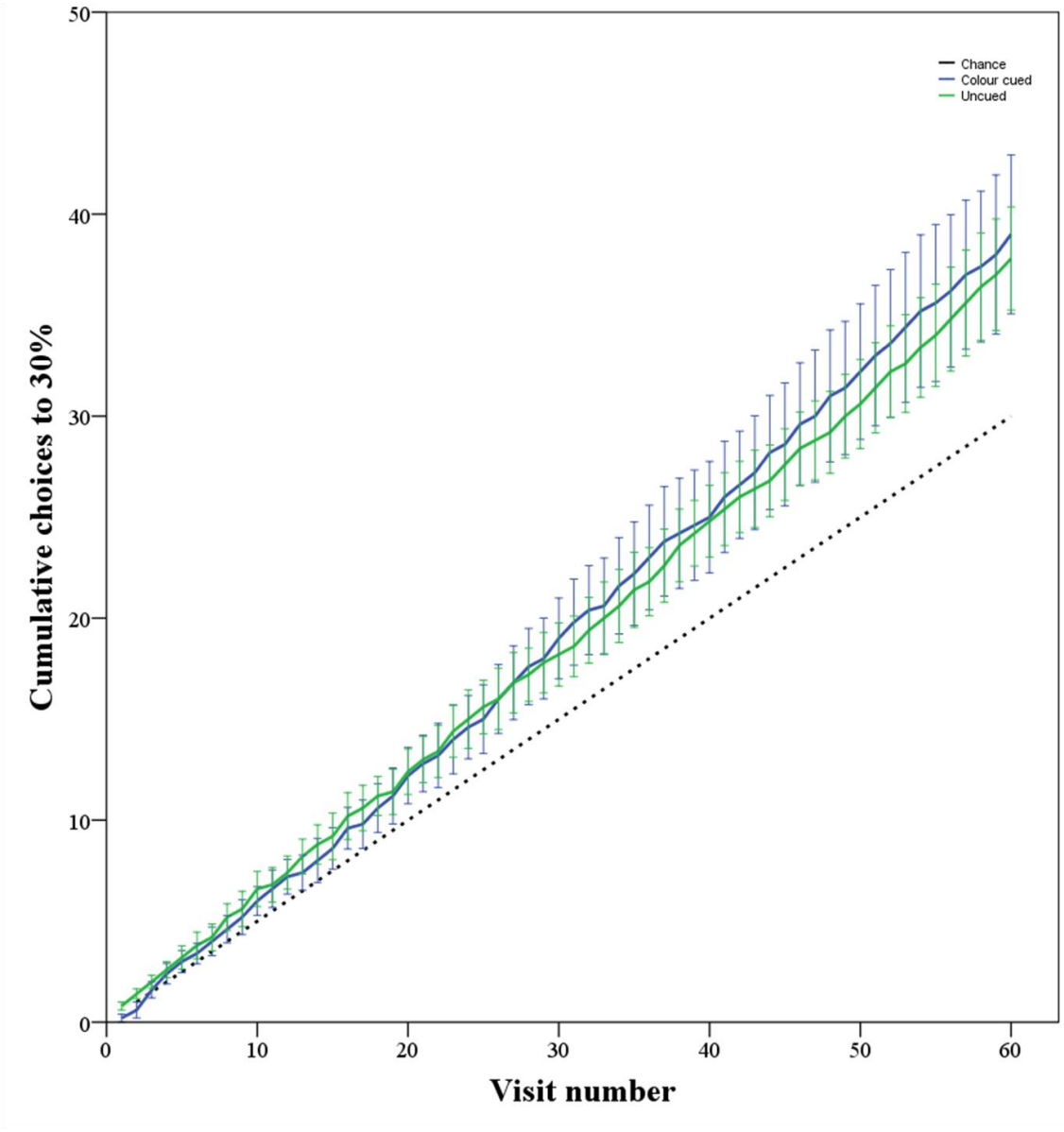

Figure 2. Concentration Treatment. *Mean cumulative visits to flowers containing 30% sucrose in the Concentration Treatment. The blue line represents the Cued condition and the green line represents the Uncued condition. The dotted black line gives chance performance. Error bars: +/- SEM. N = 5 in each treatment.*

We carried out the analysis with and without the bird that had previously participated in the Refill Treatment. As there was no qualitative difference between the results for the two tests the data presented here include all five birds. There was no evidence of an effect of treatment on the way in which a bird's preference for flowers

containing 30% sucrose changed over time (median: 0.033, CI: -0.394, 0.438) and no evidence of an effect of whether or not birds were in the Cued condition on the strength of their preference for the 30 % flowers (median: 0.019, CI: -0.322, 0.375). There was also no effect of treatment order on how preferences changed with experience (median: -0.010 CI: -0.498, 0.307), although there was some evidence that bird's preferences may have been slightly weaker on their second trial (median: -0.326, CI: -0.674, 0.008). Birds' preferences for flowers containing 30% sucrose appear to have increased slightly with visit number, although if so the effect was not strong (median: 0.156, CI: -0.236, 0.589).

Refill Treatment

The post-reinforcement pauses for each bird visiting each type of flower (10 or 20 minutes) were analysed using Mann-Whitney U-tests for each bird for each consecutive set of 50 floral visits. Seven of the eight birds tested learned to revisit 20-minute flowers significantly later than they visited the 10-minute flowers. For those birds that did learn according to this criterion, at the point of first making the discrimination, across birds, the mean return time to ten minute flowers was 11 ± 2 minutes, and mean return time to twenty minute flowers was 16 ± 3 minutes. The difference between these is significant (Wilcoxon Signed Ranks test, one-tailed: $N = 7$, $Z = 2.37$, $P = 0.009$). The bird that failed to learn was a subject in the Uncued group (UC2). Summary statistics for the first block of 50 visits in which birds discriminated between ten and twenty-minute flowers (and for the last block of testing for bird UC2) are shown in Table 1. To establish whether birds in the Cued group learned to

discriminate between ten and twenty minute flowers more quickly than birds in the Uncued group, we compared the number of blocks of 50 visits birds needed to show a significant discrimination between flower types (The bird that failed to learn was assigned the total number of blocks of 50 he had completed for this analysis. As he was a member of the Uncued group, this a conservative estimate relative to the hypothesis that birds learn faster when cued with colour). Birds that learned refill schedules cued by flower colour did not learn the refill schedule faster than did birds that had to learn each flower's refill schedule separately (Mann-Whitney U-test, one-tailed: $N = 8$, $U = 7.00$, $Z = 0.29$, P = 0.43; Figure 3).

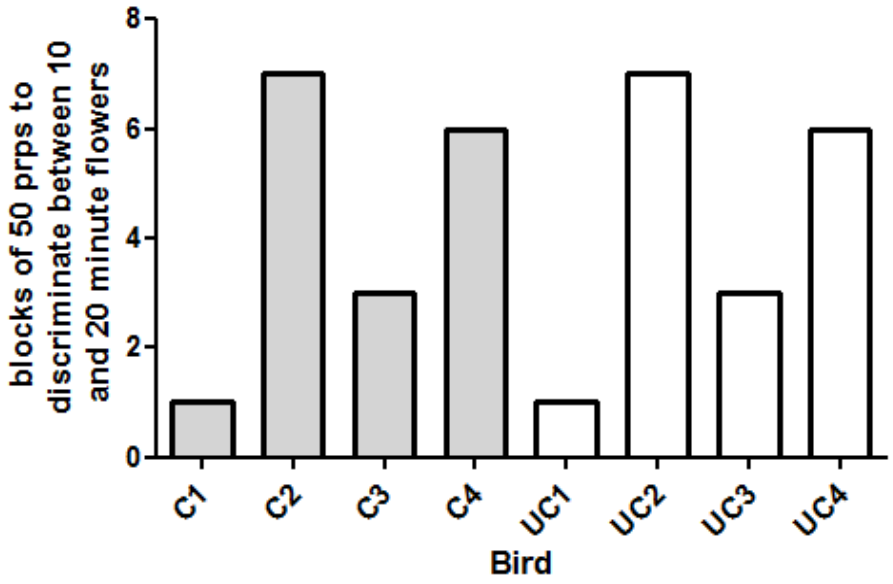

Figure 3. Refill Rate Treatment. *Number of blocks of 50 post-reinforcement pauses required by birds to discriminate between ten and twenty minute flowers in the two groups. C refers to colour cued birds, UC to Uncued birds. Bird UC2 did not discriminate between flowers by the end of training, therefore his data represent total training received.*

bird	group number	mean return time +/- S.D		U	Z	P
		10 minute flowers	20 minute flowers			
C1	1	10 ± 5, n = 32	16 ± 8, n = 18	148.00	2.84	0.002
C2	7	11 ± 4, n = 16	18 ± 7, n = 8	18.00	2.87	0.001
C3	3	11 ± 4, n = 32	15 ± 8, n = 18	202.50	1.74	0.042
C4	6	13 ± 7, n = 29	16 ± 5, n = 21	173.00	2.59	0.004
UC1	1	8 ± 15, n = 32	10 ± 4, n = 18	189.50	2.00	0.023
UC2	7	11 ± 5, n = 23	11 ± 6, n = 14	160.00	0.32	0.491
UC3	3	10 ± 6, n = 31	18 ± 10, n = 19	149.50	2.91	0.002
UC4	6	14 ± 5, n = 33	19 ± 11, n = 17	192.00	1.82	0.034

Table 1. *Summary statistics for each bird for the test block at which that bird first discriminated significantly between ten and twenty minutes or for the last block of training (for Bird UC2). U refers to the Mann-Whitney U-test. C refers to colour cued birds, UC to Uncued birds.*

Figure 4 shows running average return times to each type of flower across training for the Cued and Uncued conditions. These data, and the above analysis, suggest that condition had no effect on learning, so we developed a generalised linear mixed model with a normal error structure, modelling the log of birds' post reinforcement pauses as a function of flower type (10 or 20 minute), condition (Cued or Uncued), volume, and standardised visit number, including random bird effects on visit rate and rate of learning. We used Markov Chain Monte Carlo Methods to develop the model in WinBUGS 1.4. The model was allowed to run for 20 000 iterations across three chains, with a suitable burn-in period. The chains were thinned to remove

autocorrelations to generate 975 independent samples for each parameter. The difference in number of iterations between this simulation and that for the concentration data reflected differences in the time taken for the chains to converge between the two models and differences in the level of thinning necessary to remove autocorrelations. Both, however, gave a large number of samples on which parameter estimates are based (1237 for the concentration model and 975 for the refill rate model).

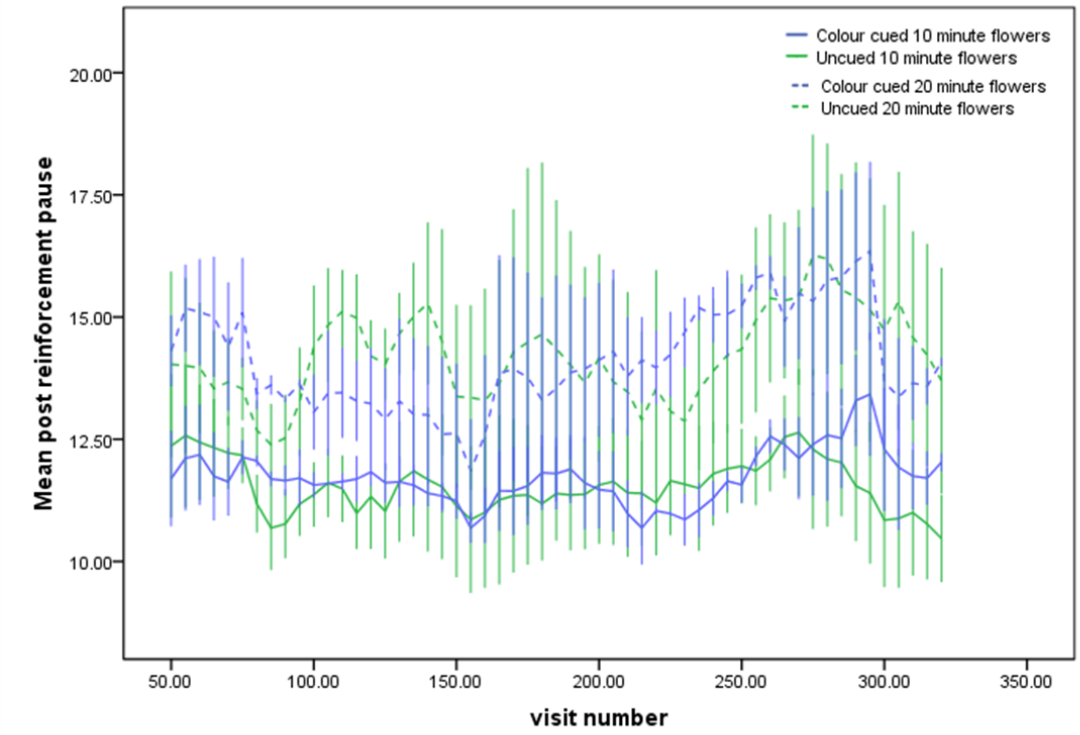

Figure 4. Refill Rate Treatment. *A plot of rolling average post-reinforcement pauses for ten and twenty minute flowers across training for both groups. Averages were calculated every ten floral visits, across blocks of 50 visits. The blue lines denote the Cued condition and the green lines the Uncued condition. Dotted lines represent twenty-minute flowers and solid lines represent ten-minute flowers. Error bars: +/- SEM. N = 4 in each treatment.*

The principal node of interest in this model was the difference between return times to ten and twenty minute flowers across training in the Cued condition compared with the Uncued condition. This was not detectably different from 0 (median estimate 5.35×10^{-4}, 95% Credible Interval (CI): -0.004, 0.002), giving a strong indication that condition had no effect on how birds' discrimination between the two flower types changed with time. There was a small effect of volume, with higher volumes corresponding to a slight increase in visit rate to both 10 and 20-minute flowers. This increase was, however, small (median 0.097, CI: 0.069, 0.128), and indicates that this technique of varying flower volume had the desired effect of keeping birds' feeding rates near constant.

Discussion

Over a very short training period (up to 30 hours), seven of the eight birds tested learned to discriminate among flowers that refilled ten minutes after they were last visited and flowers that refilled after twenty minutes. This confirms the findings of Henderson et al. (2006); that hummingbirds can indeed differentiate between intervals of this magnitude. Although birds here did not learn to the levels of accuracy seen in the Henderson et al. experiment, as they tended to return to 20 minute flowers before they had refilled, the testing period here was considerably shorter (a maximum of three days rather than ten). Some birds were also able to learn to discriminate between the flower types very quickly: one bird in each of the treatment groups had learned to discriminate between ten and twenty minute flowers within the first 50 floral visits.

Birds did not, however, learn the flowers' refill rates or their contents more rapidly if those flowers' conditions were cued by colour. This result is consistent with

the data that demonstrate that hummingbirds' prefer spatial to colour cues: if given the option of revisiting a flower in a location that was previously rewarded or of a colour that was previously rewarded, birds tend to return to the correct location (Goldsmith and Goldsmith, 1979, Hurly and Healy, 1996, Miller et al., 1985). Similarly, if presented with an array of flowers in a novel part of their territory, having experienced an array of the same shape elsewhere, birds are more likely to visit flowers in the new array which occupy the same relative position as rewarded flowers in the previous array, as opposed to flowers of the same colour (Hurly and Healy, 2002). Our data are, however, novel, in that they illustrate that, even where colour provides extra information to that provided by location, it appears that the birds do not attend to it. This is inconsistent with findings where colour appears to enhance learning of reward location. In both Miller and Miller (1971) and Healy and Hurly (1998), colour facilitated the learning of reward location by hummingbirds compared to a condition in which all of the available options are of identical colour. It may be that colour is used by birds under circumstances like those of Healy and Hurly (1998) and Miller and Miller (1971), where colour helps birds to learn locations by helping birds discriminate otherwise identical flowers from each other, but is not used to generate categories or types, which was the extra information colour provided here.

One explanation for the lack of an effect of colour on learning is that spatial information has overshadowed the cue provided by flower colour. Overshadowing can occur when two cues provide the same information but differ in their salience and is characterised by a reduced attendance to one of the cue types coupled with increased reliance upon the other. This is a ubiquitous effect, found in animals from bees to humans (Couvillon and Bitterman, 1980, Spetch, 1995) and can occur in, amongst other

contexts, spatial landmark learning (Spetch, 1995) and associative learning with auditory, visual, and taste cues (Macedo et al., 2008, Mackintosh, 1976). Which kind of cue overshadows another, however, appears to depend on the species or at least on the context. For chickadees, *Poecile gambeli*, and rats, *Rattus norvegicus*, it appears that unlike hummingbirds, colour can overshadow spatial information (Gray et al., 2005, Pearce et al., 2006), while for bees, *Apis mellifera*, odour can overshadow colour (Couvillon and Bitterman, 1982, Couvillon and Bitterman, 1980). Overshadowing relates to cue salience: generally a salient cue is thought to overshadow a less salient one (Mackintosh and Dickinson, 1979), and saliencies are likely to differ between species and circumstances. As hummingbirds will prefer spatial cues to colour cues (Goldsmith and Goldsmith, 1979, Hurly and Healy, 1996, Miller et al., 1985), location overshadowing colour in this experiment would be consistent with their wider behaviour.

As well as being consistent with apparent cue saliencies, the overshadowing of colour by space in hummingbirds may also have an ecologically plausible explanation. Flowering plants such as those the birds feed on naturally vary considerably in their nectar production rates and thus in the volume of nectar available at a given flower. This is true both for plants within the same species (Boose, 1997, Hodges, 1993, McDade and Weeks, 2004, Pleasants and Chaplin, 1983) and for different flowers on the same plant (Boose, 1997, McDade and Weeks, 2004). This variation may be a strategy by the plant to decrease sequential visits to flowers on the same plant by foragers (Biernaskie and Cartar, 2004, Biernaskie et al., 2002). Thus, if flowers of the same colour and shape vary in their reward value, the overshadowing of colour by location may not be as inefficient as it might appear at first glance, as learning about the contents

of each flower individually may sometimes be more reliable than generalising across them.

The lack of effect of colour is unlikely to be due to the birds finding the tasks too easy. In the Refill Treatment, it took the seven of eight birds that learned to discriminate between flower types an average of 189 floral visits to learn the intervals over which the two groups of flowers refilled. In the Concentration Treatment, birds' learning appears to have been far more rapid than this, although learning was no slower in the Uncued condition than it was in the Cued condition. Birds in the Cued condition were no more likely than birds in the Uncued condition to choose 30% flowers at the end of training.

These results are also unlikely to be caused by perceptual constraints. Not only have psychophysical experiments shown that black-chinned hummingbirds, *Archilochus alexandri* can see across most of the visual light spectrum (tapering off towards the red end of the spectrum: Goldsmith et al., 1981), ruby throated, *Archilochus colubris* (Miller and Miller, 1971), and rufous hummingbirds (Healy and Hurly, 1998) can, on occasion, learn more rapidly when supplied with useful colour cues, and they can learn about presence of reward (Hurly and Healy, 1996, Lara et al., 2009), reward magnitude (Bacon et al., 2010, Bateson et al., 2002, Bateson et al., 2003, Morgan et al., 2012) and variability (Hurly and Oseen, 1999) using colours similar to those we used.

In this experiment, our flowers present a very simplified version of those found in nature, varying only in one modality (colour) whereas the natural flowers a pollinator encounters are likely to also vary in other ways, such as shape and odour. The use of these different sources of information may well be complex (Leonard et al., 2011), which may aid learning as bees learn discriminations more quickly when stimuli vary in

more than one dimension (Kulahci et al., 2008), while hawkmoths *Manduca sexta* use odour to locate a flower's general location but visual cues to identify where to feed (Balkenius and Dacke, 2010). It may be that hummingbirds tend to make use of a different cue, for example scent, to categorise flowers in nature, or categorising by colour is potentiated by another feature not varied here. However, despite the relative simplicity of our flowers, this experiment still provides a compelling test of these birds' use of colour, as in similar circumstances where colour is the only floral cue and space is irrelevant, birds are able to learn colour rules.

Drawing conclusions from these results to hummingbird foraging in nature is not straightforward. Whilst, in the limited circumstances presented here, hummingbirds appear not to generalise on the basis of a type or species cue like colour, in a more naturalistic setting, where birds have to forage from many more flowers over a wider area to meet their energy demands it is quite conceivable that space may become less important and that rules about type, based on traits like colour may assume more importance. Within this investigation however, whether hummingbirds do not make use of colour because of overshadowing, floral variability, or some other cause, this experiment highlights a case where an animal does not make use of what would appear to us to be one of a resource's most obvious attributes, according to how we see the world. This apparent lack of use of colour is not due to an inability of birds to learn the type of categorical rules under investigation but rather seems to be because they do not always attend to some features of that resource. This is an important methodological point regarding testing of hummingbirds and research with animals more generally. For example, the colour of flowers in the Refill Treatment could represent the "what" dimension of a "what, where, when" test of episodic-like memory in hummingbirds.

Failure by a rufous hummingbird in such an experiment to learn about colour and refill rate might then be interpreted as a failure in birds to exhibit episodic-like memory, whereas our results suggest that such an outcome would be due to the birds attending only to the relevant cues (where the birds determine relevance, not the human experimenter). In general, then, we need to be careful to ensure that we test our assumptions about the features of a resource to which an animal attends before we draw conclusions about their cognitive abilities.

3: A field test of Scalar Expectancy Theory

Introduction

Animals are able to remember and act upon the what, where and when aspects of a past event in situations where the when of an event is specified by how long ago it happened: so-called 'episodic-like memory' (Babb and Crystal, 2006, Clayton and Dickinson, 1998, Ferkin et al., 2008, Henderson et al., 2006, Zinkivskay et al., 2009). The time scale over which these 'how long ago' judgements are tested has generally been in the order of days (Clayton and Dickinson, 1998, Ferkin et al., 2008), and rather more rarely on the scale of hours or minutes (Babb and Crystal, 2006, Henderson et al., 2006). How animals go about estimating these durations has been a source of debate: animals may assess the strength of a weakening memory trace as it decays over time (Roberts et al., 2008, Suddendorf and Corballis, 2007), they may count days (Roberts, 2002), or they may use an interval timing system (Raby and Clayton, 2009).

Of these three mechanisms (trace strength, counting, and interval timing), interval timing, in particular, has been the focus of extensive study in laboratory conditions. A wide variety of species can time short intervals, including rats, *Rattus norvegicus* (Lowe et al., 1979), pigeons, *Columba livia* (Cheng and Westwood, 1993), monkeys, *Macaca mulatta* (Dews, 1978), cats *Felis catus* (Lejeune, 1971, re-analysed in Lejeune and Wearden, 1991), fish, *Betta splendens* (Higa and Simm, 2004), bees, *Bombus impatiens* (Boisvert and Sherry, 2006) and humans (Rakitin et al., 1998). The duration tested has usually been on the order of seconds or a few minutes although there have been occasional tests over many hours (e.g. Crystal, 2001, which looks at timing up to and beyond 24 hours). From these data, two important trends are evident. Firstly, animals can estimate durations accurately over a range of intervals from seconds to

minutes (an ability sometimes referred to as mean accuracy: Lejeune and Wearden, 2006). Secondly, and most importantly for the present study, animals' ability to estimate intervals of time is Weberian in nature: that is, the error in their estimates of an interval is proportional to the interval being timed, resulting in a constant coefficient of variation over different intervals (Gibbon, 1977), and meaning that plots of the distributions of animals' estimates of different intervals will superimpose on one another when scaled to their respective means. The most popular theory to explain how animals go about making estimates of an elapsed interval is Scalar Expectancy Theory or SET (Gibbon, 1977). This postulates the existence of an internal pacemaker-accumulator system, in which a pacemaker emits pulses at a set frequency during the to-be-timed interval. These pulses are stored in an accumulator, which then compares the accumulated total to some value stored in memory (which represents the learned interval). When the accumulated number of pulses reaches the value stored in memory, the animal then starts to respond (Gibbon, 1991).

Although in explicit tests of interval timing the data are well described by SET, there are two concerns regarding the assumption that this is also the mechanism that underpins the 'when' component of episodic-like memory in tests based on "how long ago". Firstly, episodic-like studies generally make use of intervals longer than those most commonly used to study interval timing (although, as mentioned above, interval timing studies over longer intervals do exist). Secondly, studies of SET are generally conducted on animals in laboratory situations in which the animal has little to no other source of stimulation during the to-be-timed interval. How well such a system could therefore account for the behaviours and abilities of animals that, in either episodic-like

tasks or, indeed, in nature, are typically timing longer intervals as well as dealing with other demands on their attention and time, remains to be seen.

In order to investigate whether the kind of timing seen in purported episodic-like systems in any way resembles interval timing as embodied in SET, we investigated the timing abilities of wild, free-living rufous hummingbirds feeding from flowers that refilled 5, 10, 20 or 40 minutes after they had last been visited. We were firstly interested in whether birds were able to learn to time all of these intervals. Secondly, we wished to see whether the way in which these time intervals were estimated appeared to conform to the predictions of SET. In particular, we predicted that, if birds were using an SET like system they would show Weberian variance in their estimates of different time intervals, with a constant coefficient of variation across intervals, and the distributions of birds' estimates of different intervals superimposing when plotted in relative time. If timing were not Weberian, we would expect the coefficient of variation to increase with increasing interval, as longer intervals might be harder to estimate.

Methods

This study was conducted in Westcastle Valley, Alberta, Canada, at 1 400 m elevation in the Eastern Rocky Mountains, 20 km southwest of Beaver Mines (49°292 N; 114°252 W). The subjects were three territory-holding, free-living, adult male rufous hummingbirds, feeding at established territories around artificial feeders containing 14% sucrose. Birds were marked on the breast with coloured ink at the beginning of the field season so that they could be individually distinguished. Trials were run from 6:00 to 20:00 hours Mountain Daylight Savings Time from June to July, 2011.

All work was carried out under permit from Environment Canada and Alberta Sustainable Resource Development with the ethical approval of the University of Lethbridge and the University of St Andrews.

Initial Training

All birds were initially trained to feed from an artificial flower consisting of a 6 cm diameter yellow cardboard circle, with a syringe cap in the centre, forming the "nectar well", which was filled with roughly 600ul of 20% sucrose. Each flower was mounted on a 60 cm long wooden stake and presented to the bird in various locations around his territory. Training the birds to feed from this flower typically took an afternoon to complete. The following morning, this flower was placed below the feeder and the feeder removed. The flower would then be moved in increments of roughly a metre to the site of the bird's first experimental array.

Experimental Procedure

Each bird experienced four different treatments (5 minutes, 10 minutes, 20 minutes and 40 minutes) in a pseudo-randomised order that differed between birds. These different treatments all followed a similar pattern. Within a treatment, birds were initially presented with eight artificial flowers arranged in a hexagonal pattern with a nearest neighbour distance of 60 cm (Figure 1). Each flower was differently coloured or patterned. All flowers within a treatment initially contained a fixed volume of 20% sucrose (this volume varied among treatments). Once a bird had fed from one of these flowers, it would then take a predetermined length of time to refill. In the 5-minute treatment, a flower would be refilled five minutes after a bird had last fed from it, in the

10-minute treatment after ten minutes, and so on for the 20- and 40-minute treatments. When filled, flowers contained 10, 20, 40 or 80 µl of sucrose in the 5, 10, 20 and 40-minute treatments respectively. This variation in volume meant that, within the four treatments, sucrose was provided at roughly the same rate (the exact rate being determined by the birds' behaviour), and ensured that birds could meet their energetic requirements in all four treatments by feeding from a different number of flowers per visit in the different treatments.

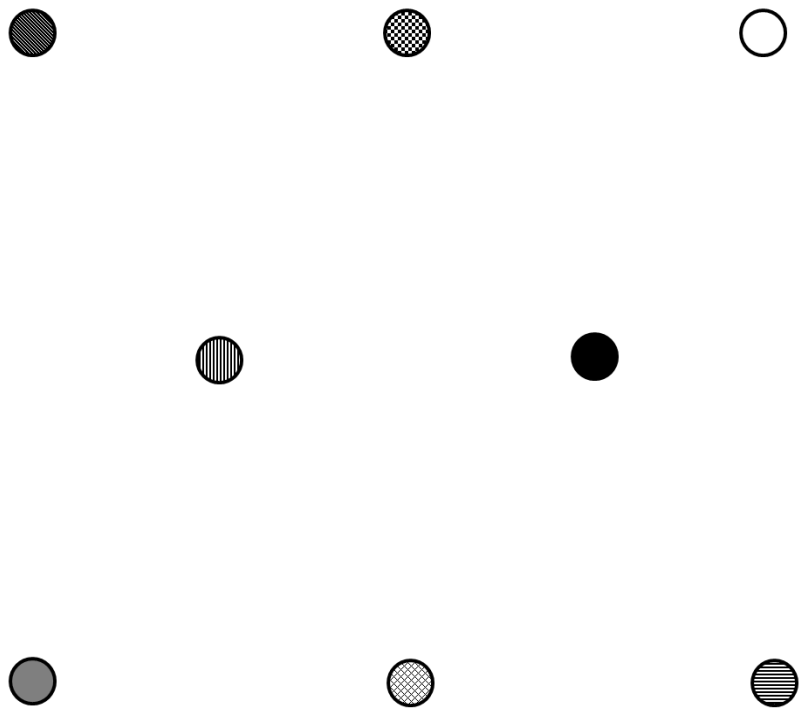

Figure 1. *Sample array used in the four treatments. Circles represent artificial flowers, nearest neighbour distances were 60 cm. Within an array, all flowers were individually distinctive.*

When a bird visited this array, the time of its visit, the flowers visited, the order of flower visits, and whether each flower was full or empty when the bird visited it were recorded. From these data, we calculated the Post-Reinforcement Pauses (PRPs) for each time a bird fed from a flower, which is how long a bird waited after feeding from a flower before returning to that flower and attempting to feed from it again. This is a typical measure of interval estimation in SET studies (c.f. Lowe et al., 1979) used in previous hummingbird timing work (Henderson et al., 2006). After a bird had visited a flower, any remaining sucrose in that flower was measured and removed. After the appropriate interval (5, 10, 20 or 40 minutes) had elapsed, the emptied flowers were refilled by the experimenter. We considered it unlikely that hummingbirds would learn to use the experimenter's movements as a cue to whether or not flowers were empty or full but to reduce that possibility, whenever a flower was refilled, the researcher sham refilled all eight flowers.

A bird was allowed to feed from the array freely until he had generated 160 PRPs, after which the array was removed and his feeder returned. After an interval of at least an hour, the bird's next treatment could begin, using a similar array of eight flowers. This array was located in a different part of the bird's territory and refilled according to a different refill schedule. Treatment arrays were always at least three metres distant from each other and at least five metres from the most recently used array location. Flowers of the same colour pattern were not used in consecutive treatments. In subsequent analysis, the last 80 PRPs for each bird in each treatment were used as an estimate of performance.

Analysis

The data were analysed using R.2.14.1 and SPSS 19. Cumulative frequency curves were constructed in GraphPad Prism 5. Mixed effects models were generated in R using the package nlme.

Results

Birds' use of the array

In the feeding bouts comprising the last 80 PRPs, birds fed from an average of 5.90 ± 2.00 flowers in the 5-minute treatment, 4.69 ± 1.70 flowers in the 10-minute treatment, 2.89 ± 1.50 flowers in the 20-minute treatment and 1.69 ± 0.85 flowers in the 40-minute treatment (all mean ± standard deviation). For these groups of feeding bouts, the interbout intervals (time elapsed between one visit to the array and the next) were 9.15 ± 10.46, 12.41 ± 4.48, 11.56 ± 7.04, 11.78 ± 6.22 minutes.

Mean Accuracy

We compared the birds' estimates of refill rates across treatments using a mixed effects model, modelling the log of PRP as a function of treatment (5, 10, 20 and 40 minutes), with bird as a random factor and using the 10-minute condition as the baseline to which PRPs in the other conditions were compared (10 minutes being the closest to birds' natural return times to flowers of this sort). The birds' performances were significantly different from their performance at ten minutes for all conditions (Model $DF = 954$, AIC = 149.34, compared to 10; 5: $T = 10.84$, $P < 0.0001$; 20: $T = 3.06$, $P = 0.0023$; 40: $T = 10.52$, $P < 0.0001$). However, their performance did deviate from strict matching. Specifically, birds seemed to overestimate return times at 5, 10 and 20

minute intervals and to underestimate return times to 40 minute flowers (see Figure 2 for the last 80 post-reinforcement pauses for birds feeding from 5, 10, 20 and 40-minute flowers).

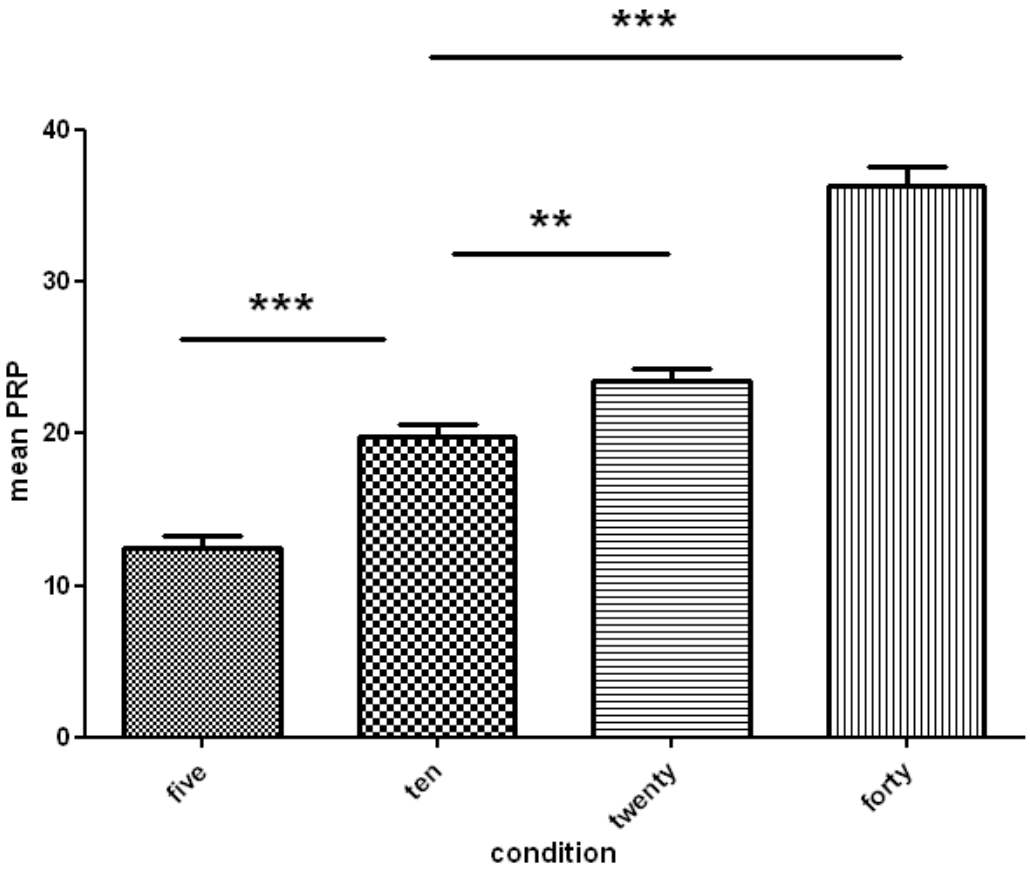

Figure 2. *Bar chart of birds' estimates of refill times in each of the four treatments for the last 80 PRPs of training. N = 240 for each bar. *signifies p < 0.05, ** indicates p < 0.01, *** indicates p < 0.001.*

Because sucrose volumes varied across treatments, we wished to establish that the difference in birds' return times across conditions was due to the birds remembering how long ago they had visited a flower, rather than merely due to variation in the number of flowers from which a bird had to feed on a given visit to meet his energy

demands. We therefore conducted an analysis to explore how much the PRPs shown in Figure 2 truly reflect a memory for how long ago each flower had been visited. On a given foraging bout, a bird only visited a selection of the flowers available, which meant that the PRPs generated were a subset of those that could be generated at a given time. In order to assess whether birds' performances in the last half of training represented actual timing of the intervals, for each foraging bout we calculated the average PRP of the flowers birds visited and compared this to the average PRP of the flowers not visited during that bout. If the birds were timing individual flowers they should avoid those they had visited most recently and, therefore, the PRP of flowers visited should be consistently longer than the PRP of those not visited. We found that, for all three birds, the PRP for flowers visited did not differ from that for those flowers not visited in the 10-minute condition. For two of the three birds there was also no difference in the 5-minute condition. For the third bird, the difference the PRPs to visited flowers were significantly longer than to the flowers not visited to the 0.05 level at 5 minutes (see Table 1 for summary statistics). In contrast, for all birds, flowers visited had significantly longer PRPs than those not visited in the 20 and 40 minute treatments (Table 1, Figure 3).

Bird	Treatment	*N*	*Z*	*P*	*R*
Bird 1	5	17	-3.153	**0.002**	0.541
	10	12	-0.866	0.386	0.177
	20	31	-3.547	**<0.001**	0.450
	40	46	-4.491	**<0.001**	0.468
Bird 4	5	7	1.069	0.285	0.286
	10	19	-0.327	0.744	0.053
	20	32	-3.988	**<0.001**	0.499
	40	41	-4.892	**<0.001**	0.540
Bird 23b	5	9	-1.185	0.236	0.279
	10	15	-0.094	0.925	0.017
	20	16	-2.135	**0.033**	0.377
	40	29	-2.499	**0.012**	0.328

Table 1*: Results of Related Samples Wilcoxon Signed Rank tests comparing the PRPs of visited and unvisited flowers for each bird in each treatment.*

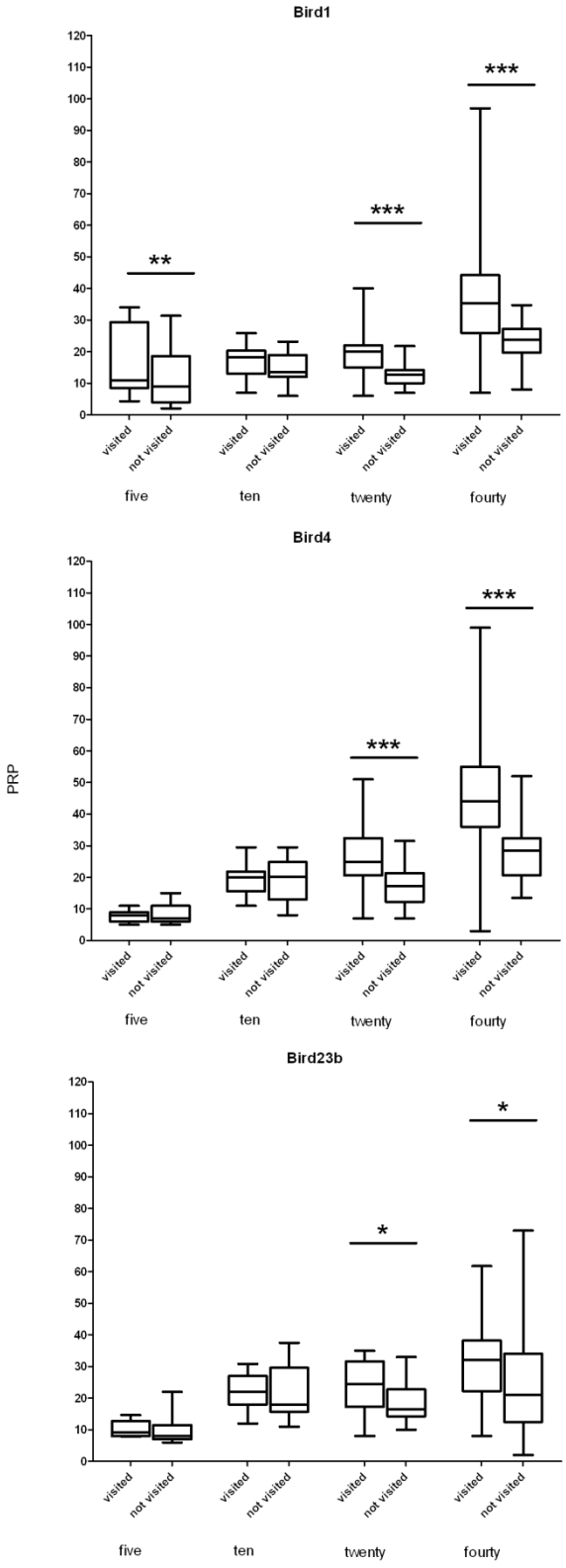

Figure 3*. Box-plots of the average PRPs of flowers visited versus not visited in a bout for each bird in each condition. A significant difference between visited and unvisited flowers in a treatment indicates birds are discriminating between flowers in a feeding visit. Boxes represent means with interquartile ranges, whiskers give ranges. Sample sizes for visited and not visited flowers are given below each condition. *signifies $p < 0.05$, ** indicates $p < 0.01$, *** indicates $p < 0.001$. Sample sizes for each bar are given in table 2.*

As an additional assessment of the birds' performance, we looked at how birds' PRPs changed over training. If the birds learned about a flower's refill schedule, we would expect PRPs to increase across training, as this would reduce the chance of visiting an empty flower. As a model including all treatments for all birds failed to converge, we looked at each treatment individually. We used mixed-effects models for each condition, modelling log of PRP as a function of visit number, with a random effects of bird on the model intercept and visit number. PRPs increased across training for the 10, 20 and 40-minute conditions (10 minutes: model DF = 476, AIC = -73.22, visit T = 3.39, P < 0.001; 20 minutes: model DF = 476, AIC = 12.05, visit T = 3.47, P < 0.001; 40 minutes: DF = 476, AIC = 127.48, visit T = 4.97, P < 0.0001). It was not possible to model the data for the 5-minute treatment in the same way, so for each of the three birds we conducted a separate linear regression of log PRP against visit number. For two of the three birds there was no significant change in PRP as visit number increased, however, for the third bird there was a small but significant increase in PRP with training (Bird 1: Adjusted R^2 < 0.001, T = 0.20, P = 0.838; Bird 4: Adjusted R^2 < 0.001, T < -0.01, P = 0.997; Bird 23b: Adjusted R^2 = 0.098, T = 4.15, P < 0.001).

Scalar variance

Whilst the number of PRPs per bird was large, the number of birds was small, so we investigated the property of scalar variance both within and between subjects. Following convention, we converted PRPs for each bird at each interval into relative time. However, as birds' mean performance did not match the refill schedule in most cases, we used a bird's own mean estimate of an interval, rather than the real interval as the scaling factor. We then plotted superimposed cumulative frequency distributions for

each bird. Conventionally, when curves are plotted in relative time in this way, frequency distributions are used rather than cumulative frequency distributions (Lejeune and Wearden, 2006). However, we used cumulative distributions because this removed the need to specify bin width, which might introduce an extra source of error given the relatively small size of our data set. In these plots, we would expect the point of inflection of each line to be at 1 on the x-axis, as this corresponds to the mean return time for each condition. The similarity or otherwise between the distributions can then be judged from the relative shape of the curve to either side of this line: if two lines superimpose across relative time, then the distributions will lie on top of one another. A line that is steeper either side of the point of inflection represents a distribution that is tighter around the mean (i.e. has a lower coefficient of variation. A line that is flatter, on the other hand, represents a source distribution with a higher coefficient of variation. As can be seen in Figure 4, the curves for the 10, 20 and 40-minute treatments showed high levels of overlap for all birds, whilst the curve for 5 minutes did not. There do not appear to be any systematic differences between the curves for the 10, 20, and 40-minute distributions. When the data are aggregated together across all three birds, there is considerable overlap for the curves for 10, 20 and 40 minutes, but not for 5 minutes. To assess whether the ten, twenty, and forty minute curves were statistically different from each other, we conducted two sample Kolmogorov-Smirnov tests for each bird to compare the distributions of birds' PRPs in relative time between 10 and 20-minute flowers, and 20 and 40-minute flowers, and 10 and 40-minute flowers, using a significance level of 0.025 to correct for testing each data set tested against another twice. The only significant difference between distributions was between the 10 and 20-minute treatments for bird 23b, although the differences between the 10 and 40-

minute treatments were also near significant for this bird, as was the difference between the 10 and 20–minute treatments for bird 4 (Table 2). These differences appear to have been driven by high levels of positive skew in the 10-minute treatment for bird 23b, and to a lesser extent for bird 4.

As well as looking at frequency distributions to assess the scalar property of animals' estimates of different intervals, the coefficients of variation of animals' estimates can be compared. This results in far fewer data per subject, but gives the same general trend (Figure 5). The coefficients of variation of birds' individual return times seem to be conserved at 20 and 40 minutes, somewhat ambiguous at ten minutes, and highly variable at 5 minutes.

Bird	intervals compared	*D*	*P*
4	10-20	1.27	0.082
	10-40	1.19	0.12
	20-40	0.87	0.836
1	10-20	0.79	0.56
	10-40	0.71	0.692
	20-40	0.79	0.56
23b	10-20	1.82	**0.003**
	10-40	1.34	0.054
	20-40	0.95	0.329

Table 2. *Results of two-sample Kolmogorov–Smirnov tests comparing post reinforcement pauses in relative time between each condition for each bird. As each data-set is tested twice, the significance level used here is 0.025. N= 160 in all cases.*

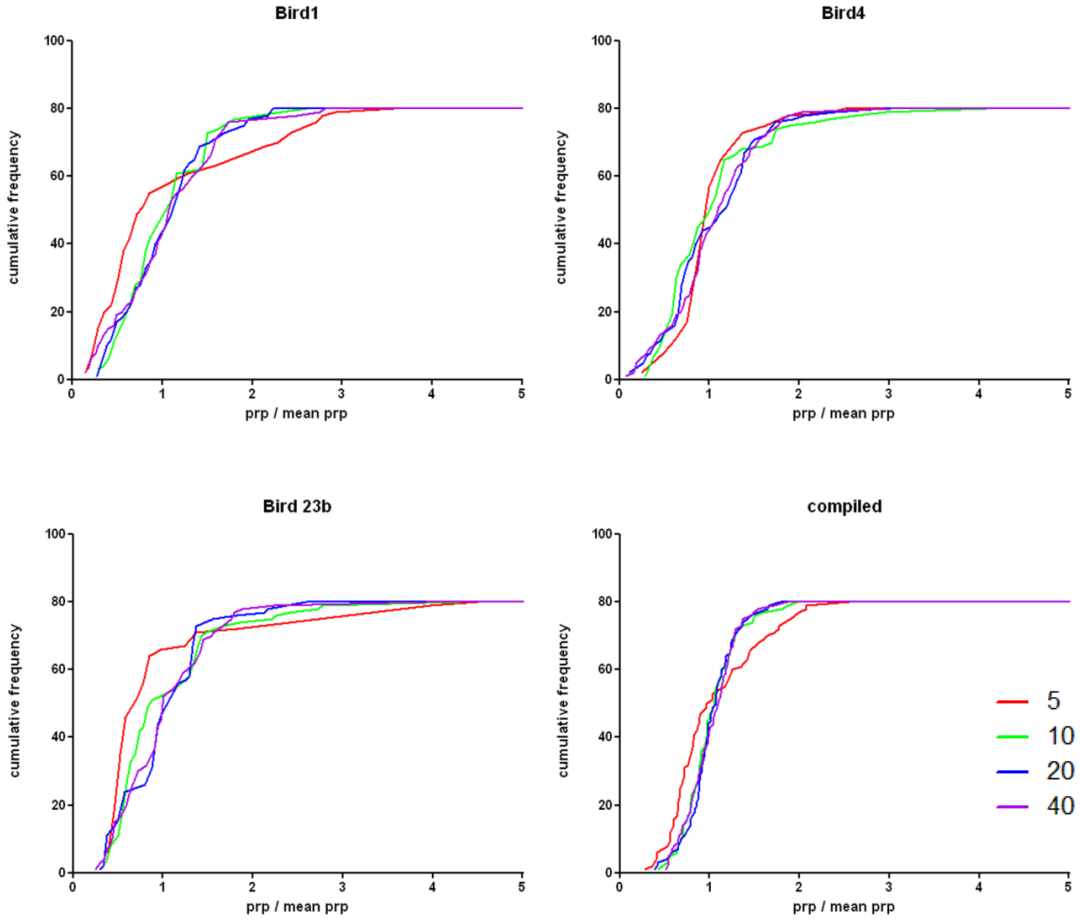

Figure 4. *Cumulative frequency distributions for birds' estimates of PRPs relative to the mean in each treatment. For each treatment, "1" on the x-axis corresponds to a birds' mean estimate of the interval being timed, numbers less than 1 are underestimates and numbers greater than 1 are over estimates (relative to the bird's average PRP, not the interval itself). The panel labelled "compiled" shows the mean curves for each condition across birds. Red lines indicate the 5-minute condition, green lines the 10-minute condition, blue lines the 20-minute condition, and purple lines the 40-minute condition. N=80 for each bird in each treatment.*

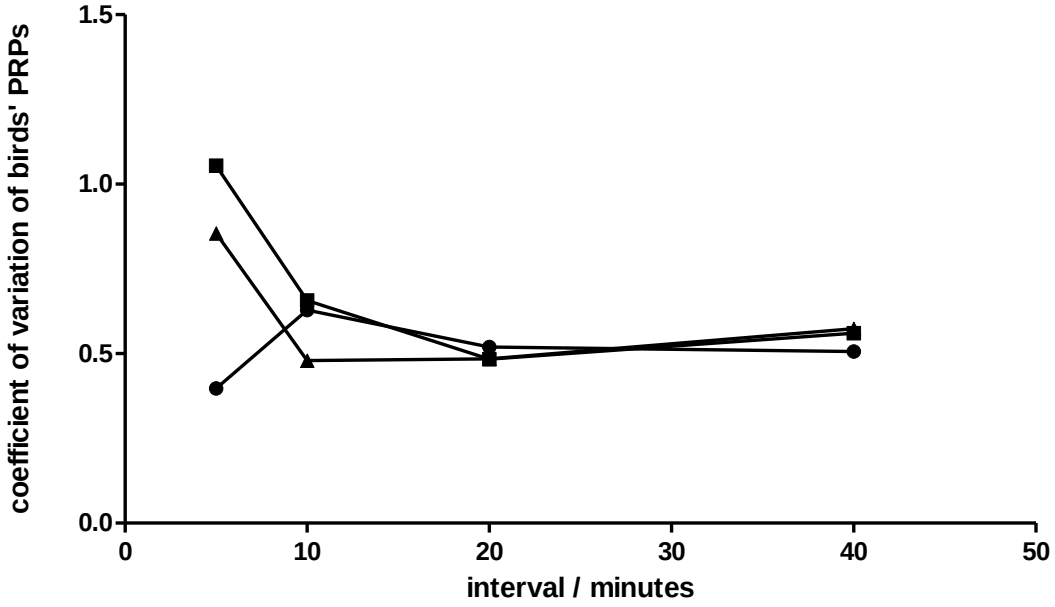

Figure 5*: Coefficients of variation for each bird at each interval. Data for bird 23b are given by squares, bird 1 by triangles and bird 4 by circles.*

Discussion

Birds could distinguish among the four time intervals and revisited the different arrays according to flowers' refill schedules (Figure 2), indicating that hummingbirds can time intervals twice as long as previously demonstrated (Henderson et al., 2006). Further, this timing in the case of 20, 40, and to some degree 10, minute intervals appears to have been Weberian in nature, conforming to one of the central tenants of Scalar Expectancy Theory (Gibbon, 1977, 1991, see Figures 3 and 4). Whilst Scalar Expectancy Theory has been applied to natural behavioural analogues within laboratory situations in a limited number of contexts, such as patch departure times at different rates of reinforcement (and using intervals of the order of seconds: Kacelnik and Brunner, 2002) or time-place learning (Carr and Wilkie, 1998, Pizzo and Crystal, 2004), this is, as far as we are aware, the first demonstration of scalar timing in the wild, and presents the first evidence that the rules found to govern interval timing in laboratory

situations may extend to the problems facing free-living animals. Although we were only able to test three birds in this experiment, due to time constraints, we were able to gather a sizeable amount of data per bird (160 PRPs for each bird in each condition), and have conducted the majority of these analyses on a bird by bird basis, therefore we contend that these results are fairly robust for the birds we studied.

Whilst the birds' performance reflects the central Webarian property of SET, our results do show some departures from classical Scalar Expectancy Theory. Typically, timing studies using PRPs find that the relationship between PRP and elapsed time is best described by a power-law relation with a fractional exponent, such that the relationship between estimated and elapsed time flattens out at longer intervals (for a review see Lejeune and Wearden, 2006). Here, the trend is less straightforward, with birds overestimating 5, 10 and 20-minute intervals, and slightly underestimating 40-minute intervals (see Figure 2). It seems plausible however that this may be a result of the testing situation. In the context of a laboratory test, the only activity the test animal is engaged in is the interval timing task the experimenter presents it with. The wild, free-living hummingbirds used here, on the other hand, were engaged in a range of other behaviours, including territory defence and mating efforts. Time allocation among these other activities could have altered the pattern of time estimates across intervals. Hummingbirds also practice defence by exploitation (Garrison and Gass, 1999, Gill, 1988, Paton and Carpenter, 1984) and it is likely that this would cause differences in birds' preferred return times to flowers taking different lengths of time to refill to different volumes: there is a large energetic difference between allowing the contents of a flower that takes 10 minutes to refill to a volume of 20 µl to be pilfered and allowing

the contents of a flower takes 40 minutes to refill to 80 µl to be pilfered. How birds' time estimates represent a trade-off between these different factors requires future study.

The differences in return time to flowers refilling after 10, 20 and 40 minutes (Figure 2), and the Weberian relationship between these time estimates, appear to be the result of animals timing these intervals, rather than being driven by the slight differences in experimental procedure between these conditions. In all of these conditions, birds increased their PRPs over training, demonstrating that birds were learning about flowers' behaviour, as by tending to wait longer to return to a flower, birds decrease their probability of returning to that flower whilst it is still empty. In the case of 20 and 40-minute intervals, additional strong evidence for timing is provided by the comparison of flowers that were or were not visited in a given bout, with all three birds preferring to visit flowers that had been left longer to refill (Table 1). This within-bout discrimination was not detectable in the 10-minute treatment, where no bird discriminated between flowers significantly. Whilst the effect sizes for this treatment are very small, it is important to note that there were fewer data in this treatment due to birds visiting more flowers per bout (hence requiring fewer bouts to complete training; Table 1). In addition, because we expect flowers to be visited more often in this condition, the difference in elapsed time since last visit between selected and unselected flowers is likely to be smaller. This means the smaller sample size caused by birds visiting more flowers in a bout is being used to detect what we would theoretically expect to be a weaker effect. Furthermore, a previous experiment showed that these hummingbirds can time 10-minute intervals (Henderson et al, 2006).

In contrast to the 10, 20, and 40-minute conditions, the majority of the evidence available suggests the birds did not time flower refill times in the 5-minute condition.

From the start of training, birds' probability of visiting an empty flower was very low in this treatment (an average of 18.7 of birds' first 20 flower visits in this condition were rewarded, compared to 16.0, 11.0, and 11.3 in the 10, 20 and 40-minute treatments). Essentially, as birds' return times to the array were generally much longer than were individual flowers' refill schedules (their average return time was 9.45 minutes in the last half of training), it seems unlikely that in this treatment birds would have needed to keep track of the times of their visits to individual flowers.

On potential alternative explanation of birds' behaviour in this experiment is that birds are able to detect which flowers have sucrose in them before sampling, or learn to do so over training. As mentioned earlier, previous work has shown these birds are unable to detect sucrose in flowers without sampling (Hurly, 1996). We also think it is extremely unlikely that, across the training periods used here (the longest in this thesis, and amongst the longest used in this study system in general) these birds have learned to detect whether flowers are full or not without sampling. This is borne out by the high number of visits birds make to empty flowers in conditions where flowers are often empty. Of the last 50 flower visits made by birds in the twenty minute and forty minute conditions, 30-34% and 40-48% of visits were to empty flowers respectively. Further, even at the end of training, the majority of birds' first revisits to 40 minute flowers occurred before they were full: 48-73% of PRPs in the trained stage were less than 40 minutes, hence corresponded to visiting empty flowers, despite birds having learned to avoid flowers they had recently emptied (see Figure 3 and associated analysis). The bird who performed worst (that is, made 73% of his last 80 PRPs to empty flowers) experienced the 40 minute condition at the end of training, therefore had had the most opportunity to learn any cues available from the sucrose. That most PRPs for this

treatment underestimate floral refill times, despite training, and that more visits to empty flowers in the last 50 visits of training occurred in the 40 minute condition than any other, despite these flowers containing more sucrose, hence the potential to produce a greater signal, suggests strongly that detection of sucrose via visual cues (sucrose is not volatile, does not produce olfactory cues) is not responsible for the results seen here, and that these flowers do not provide cues that birds can learn to use to direct their foraging. These results are therefore, we contend, a result of birds attending to the refill times of flowers.

We have framed the discussion of interval timing in this study in terms of SET, as this is currently the most prevalent theory in laboratory studies of interval timing. However, it is worth noting that there are competing theories of animal timing. These include the Oscillator Model (Gallistel, 1990), which proposes that the interval timing system is controlled by a number of oscillators, similar to the circadian timing system, but with different periodicities, the Behavioural Theory of Timing (BeT: Killeen and Fetterman, 1988), which proposes that intervals are timed by animals moving through a stereotyped sequence of states or behaviours, with the behaviour corresponding to the end of the interval being timed becoming associated with a reward, and the Multiple-Time-Scale (MTS) theory, which states that intervals are timed by assessing the strength of a memory for an event as it decays, and using this to infer an elapsed duration (Staddon and Higa, 1999). These alternatives, particularly the former, are becoming increasingly popular (Shettleworth, 2010), and they can all also predict the scalar property we found. We cannot rule out an oscillatory mechanism for accounting for these results although we do not observe areas of lower variance are predicted by this theory (Crystal, 2001), but we think the behavioural theory of timing is unlikely to

account for these data, and for timing in the wild more generally. BeT postulates that animals measure time by performing a stereotyped programme of movements or actions with highly repeatable durations and that this sequence of behaviours can, therefore, be used to estimate an interval. However, the wild birds we studied were engaged in varied and unpredictable behaviours throughout training, such as chasing intruders, hawking for insects and displaying for (and mating with) females as the opportunity arose. Against this varied behavioural backdrop, it seems most unlikely that a system that relied upon repeated behaviour would support the reliable timing we observed.

Interval timing systems have been suggested as an alternative mechanism by which what, where, when, tests can be solved without the need for episodic like memory (Raby and Clayton, 2009), and our results suggest that, over the timescales we used, this is a real possibility. Although our testing paradigm was not episodic-like in the strictest sense, as birds received repeated trials in the same location (so the what, where and when are not trial unique: Clayton et al., 2003a), our test scenario did require animals to be aware of what, where and when to organise their foraging efficiently. As birds can use similar rules when trials are unique (González-Gómez et al., 2011), we believe that our data can inform on this debate. When interval timing is mentioned in relation to episodic-like memory, the implication seems to be that interval timing is an alternative explanation for the behaviour observed (Raby and Clayton, 2009). However, we would contend that the situation is somewhat less straightforward. For an animal to respond appropriately to the what, where and when of an event, it must have some mechanism for encoding "when", and it seems to us that the interval timing system may often by usefully co-opted to this function. The interval timing system is dependent on a wide range of brain areas, including the hippocampus (for a brief review see Meck,

2005), which is thought to be the area responsible for episodic (Tulving, 2002), and episodic-like memory (Fortin et al., 2002). Furthermore, the amnesiac patient HM, the subject of many classic studies of episodic memory impairment, was also impaired at interval timing tasks (Richards, 1973). Scalar Expectancy Theory and the Behavioural Theory of timing are prospective models, in that a duration is estimated from a process which starts at the beginning of the timed interval, and then continues to a point that an animal has learned to associate with a reward (either a value stored in an accumulator in the case of SET, or a behaviour in the case of BeT) and hence no memory for the event that started the interval timer is necessarily required for it to produce timing. However, the Oscillator model (Gallistel, 1990) has more in common with circadian timing (such that it has been argued that circadian timing is a special case of interval timing: Crystal, 2001), and circadian rules have been argued as useful forms of time for episodic-like tasks (Roberts et al., 2008). The Multiple-Time-Scale model (Staddon and Higa, 1999), on the other hand, relies on memory decay as a method of estimating how long ago an event happened, and thus, by definition, requires some memory of a past event. Until we have reached a consensus on which of these models of interval timing is correct, we think that efforts to determine whether memory is truly episodic, and how closely it resembles human episodic memory, would be more usefully directed at other questions relating to the similarities and differences between human and animal memory, such as how the different aspects of memory are integrated together to guide behaviour (Schacter et al., 1998).

Chapter 4: Can hummingbirds detect rates of change?

Introduction

Recent work in the field of episodic-like memory and time-place learning has demonstrated that animals can learn when to expect a reward. For example, scrub jays *Aphelocoma coerulescens* can learn the duration over which a food item will have decayed: when taught that a preferred food is perishable after a long delay between storage and recovery, but not a short delay , birds will preferentially search for a preferred food after a short delay but not a long one (Clayton and Dickinson, 1998). Similarly, meadow voles *Microtus pennsylvanicus* can remember how long ago they encountered a female in different stages of oestrus (Ferkin et al., 2008) and hummingbirds *Selasphorus rufus* can learn experimental flower refill intervals (Henderson et al., 2006). Furthermore, animals can also use other kinds of timing to guide their behaviour, such as time of day (Pahl et al., 2007), an item's place in a sequence (Fortin et al., 2002), or the context in which an event occurred (Eacott and Norman, 2004).

In the vast majority of these studies, the challenge the animal faces is to judge whether or not food is available at a given time. This has been described as a problem of determining durations before ripening, decay (Clayton and Dickinson, 1998) or replenishment (Babb and Crystal, 2006). When we train animals to these procedures we generally use an all-or-nothing approach: food is either present or not, fresh or inedible and its state changes as a step function. However, in the real world, changes of this sort tend to occur gradually over time.

Exploiting a resource that changes gradually may present different challenges to exploiting one that changes as a step function. One effect particular to gradual changes

of state may be that animals are less likely to encounter resources that are completely unrewarding and more likely to encounter resources that offer part of the possible total reward. This may mean that estimating the appropriate time to return to a resource is more difficult. For example, many animals appear to be poor at inhibiting their response to a stimulus that will produce a small reward now, versus a larger one later (Dufour et al., 2007, Paxton and Hampton, 2009).

Nectarivorous species may be particularly useful for addressing questions of how animals treat resources that change their state slowly, as the flowers from which they feed replenish gradually after being emptied. In particular, the rufous hummingbird, *Selasphorus rufus*, may be useful. These birds can learn flower refill times when those flowers refill discretely (Henderson et al., 2006, Marshall et al., 2012). We do not, however, know how they view flowers that refill more slowly. Although Garrison and Gass (1999) found that the long-tailed hermit hummingbird *Phaethornis superciliosus* decreased its use of feeders throughout the day as nectar production decreased across the day, this daily pattern of change in feeding rate may either be due to the birds' being able to detect a difference in nectar delivery rate or to the birds having a daily pattern to their foraging (Gass and Garrison, 1999). Garrison and Gass also varied sucrose delivery rates between flowers, to allow faster and slower producers of sucrose to be compared. The effects of this treatment were, however, mixed, with birds attending to increases in rate but not decreases, and the data collected do not easily allow detailed comparison of refill and return rates between flowers. How hummingbirds respond to different rates of change, and how closely their return times to different flowers reflect differences in filling rates thus remains an open question.

To determine, then, whether hummingbirds can track gradual changes in the rates of reward delivery, we presented five wild, free-living hummingbirds with an array of eight flowers. To four of the flowers we added 5µl of sucrose every five minutes and to the other four we added 10µl every five minutes. Based on their ability to differentiate between resources that refill discretely at different intervals, we predicted that hummingbirds would be able to detect the different refill rates of these two types of flowers, and would visit the more rewarding flowers more often than the less rewarding ones. In particular, we predicted that birds would be expected to visit more rewarding flowers twice as often as the less rewarding ones, as they fill twice as quickly as less rewarding flowers. This is a strong form of the Matching Law, which states that animals revisit options in a manner proportional to their payoffs and which has been used in its generalised form to investigate foraging in the field (e.g. Houston, 1986) and in laboratory situations (reviewed in Williams, 1988, although the experimental procedure generally used in the laboratory does not exactly mirror that used here). It is also possible that the birds could use the volume of reward the flower contains rather than its refill rate to determine which flowers to visit as flowers that refill twice as often as other flowers, if unvisited, will contain twice the volume of slower refilling flowers. In Experiment 2, therefore, we investigated how the hummingbirds would respond to flowers that varied in reward value but did not vary over time. Birds were again presented with an array of eight flowers, four of which contained 10µl of sucrose and four of which contained 20µl.

Methods

Subjects

This study was conducted in Westcastle Valley, Alberta, Canada, at 1 400m elevation in the Eastern Rocky Mountains, 20 km southwest of Beaver Mines (49°21' N; 114°25' W). The subjects were six territory-holding, free-living, adult male rufous hummingbirds, feeding at established territories around artificial feeders containing 14% sucrose. Birds were marked on the breast with coloured ink at the beginning of the field season so that they could be distinguished without recapture. Trials were run from 6:00 to 20:00 hours Mountain Standard Time from June to July, 2011. All work was carried out under permit from Environment Canada and Alberta Sustainable Resource Development and with the ethical approval of the University of St Andrews and the University of Lethbridge Animal Welfare Committee.

Initial Training

All birds were initially trained to feed from artificial flowers consisting of a 6cm diameter yellow cardboard circle, with a syringe cap in the centre, forming the "nectar well", which was filled with roughly 600ul of 20% sucrose. Each flower was mounted on a 1 m long wooden stake. This stage of training usually took an afternoon to complete. The following morning, this flower would again be placed below the feeder, the feeder removed, and the flower would be moved in increments of roughly a metre to the site of the experiment.

Experiment 1

Five birds were each presented with an array of eight flowers, arranged in a hexagonal pattern with a nearest neighbour distance of 60cm (Figure 1). Each flower was a different colour (red, orange, green, blue, teal, lilac, fuchsia or pink). To four of these flowers, we added 5µl of 20% sucrose every five minutes ("slow" flowers) and to the other four, we added 10µl every five minutes ("fast" flowers), up to a maximum of 120µl per flower. When the bird visited this array, the time of the visit, the flowers visited and the order in which they were visited were recorded. From these data we then calculated the inter-visit interval to each flower, giving us a measure of the time birds gave flowers to refill between visits. Each bird was allowed 28-30 hours experience of the array, which corresponded to at least 250 flower visits. As rain water could enter the flowers and noticeably change their contents, the experiment was interrupted whenever it rained and resumed as soon as possible when the rain stopped. We did not include these interruptions as part of the experience of the array. The position of flowers of the two types, and the colours of these flowers, were pseudo-randomised across birds, such that no bird experienced a situation in which all flowers of the same sort were adjacent to one another.

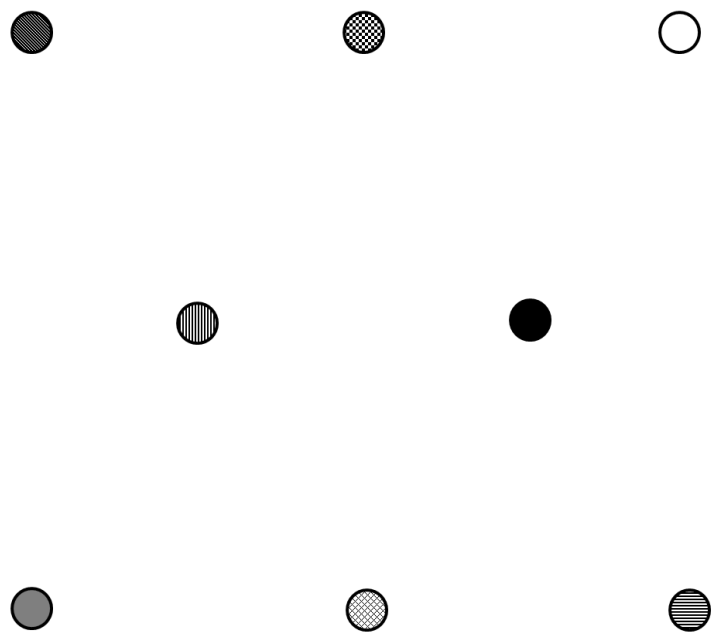

Figure 1*: Diagram of the array used in Experiments 1 and 2. The circles represent artificial flowers. Nearest neighbour distances were 60 cm. Within an array, all flowers were different colours. In Experiment 1, 4 of the 8 flowers received 5 µl of sucrose every 5 minutes and 4 received 10 µl every 5 minutes. In Experiment 2, 4 flowers contained 10 µl of sucrose, and 4 contained 20 µl of sucrose.*

Experiment 2

Four birds, three of which had previously participated in Experiment 1, were presented with an array of eight different flowers, each of which was visually distinctive. Four contained 10µl and four contained 20µl of 20% sucrose. Birds were allowed to visit this array for 8-10 hours, during which the time of each visit, and flowers visited, were recorded. Flowers were refilled manually by the experimenter after a bird had visited them. As in Experiment 1, the positions of flowers of the two types, and the appearance of these flowers, were pseudo-randomised across birds, such that no bird experienced a situation in which all flowers of the same sort were adjacent to one another.

Results

Experiment 1

Birds' preferences

To look at the birds' choices of fast and slow flowers over time, we constructed a mixed effects model with a binomial error structure, modelling flower type visited as a function of visit number, with a random effect of bird on both the model intercept and the effect of visit. There was no effect of visit number on flower type chosen (Model $N = 1795$, AIC = 2452, $Z = 0.32$, $P = 0.752$) although birds did visit fast flowers significantly more often than they visited slow flowers, as the model intercept was significantly different from 0 (which would indicate indifference between the two flower types, model intercept: 0.302 ± 0.087, $Z = 3.48$, $P < 0.001$). As birds did not change their likelihood of choosing a fast flower rather than a slow flower across training, we collapsed birds' choices across training to yield a proportion of choices to slow flowers. We then compared these preferences to a test proportion of 0.333, as this proportion is that which would be predicted under matching if birds revisit the flowers that refill twice as fast twice as often. Birds chose the slow flowers significantly more often than a third of the time (Two-tailed Wilcoxon signed rank test, $N = 5$, $Z = 2.02$, $P = 0.043$; Figure 2).

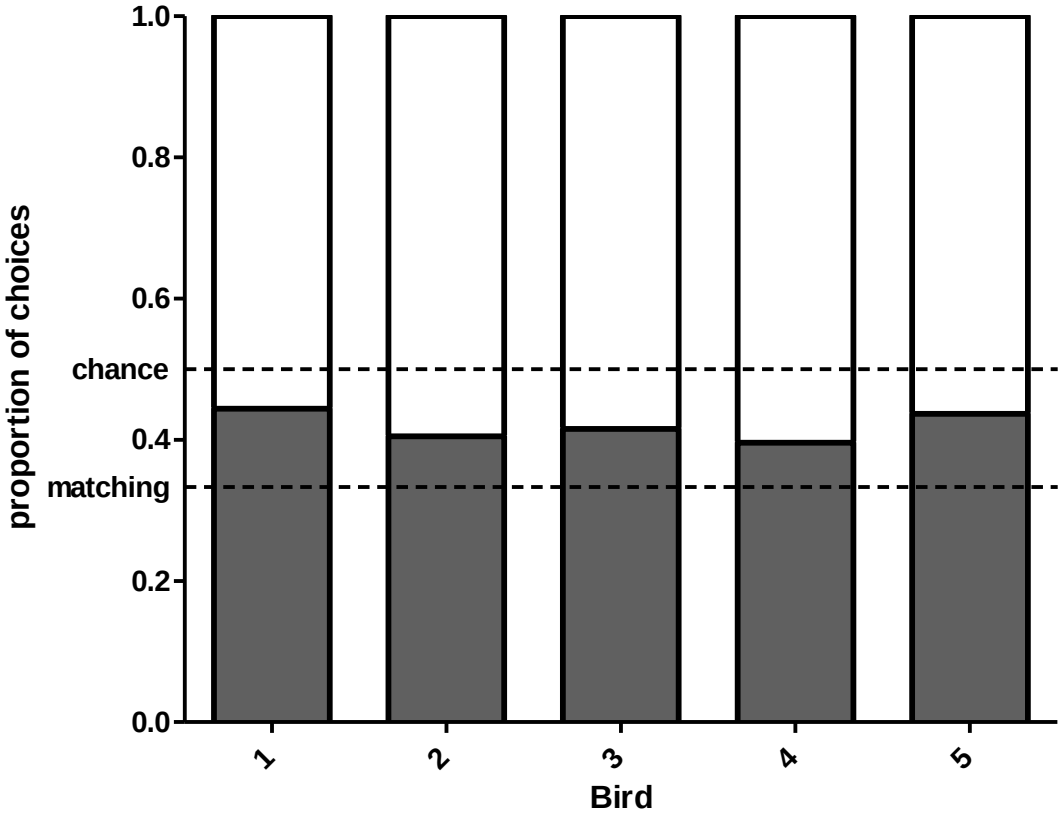

Figure 2*: Proportion of choices to slow and fast flowers made by each bird across training in Experiment 1. The open bars denote fast flowers and the grey bars denote slow flowers. The dotted lines give expected proportions under chance and under strict matching.*

Refill times

To analyse the birds' return times to the two different types of flowers, we constructed a linear mixed effects model, modelling log of inter-visit interval to individual flowers as a function of flower type, visit number, and the interaction between them, with a random effect of bird on the model intercept and visit number. As the interaction between flower type and visit number was significant (Model *DF* = 1601, AIC = 1381.68, interaction $T = 2.10$, $P = 0.036$), indicating that the inter-visit intervals to slow and fast flowers changed differently across training, we analysed the

effects of visit on both slow and fast flowers separately. For both slow and fast refilling flowers, revisit intervals to individual flowers increased significantly with training (slow flowers: Model *DF* = 657, AIC = 664.42, visit *T* = 3.43, *P* < 0.001; fast flowers: Model DF = 940, AIC = 730.69, visit: *T* = 2.51, *P* = 0.012; Figure 3).

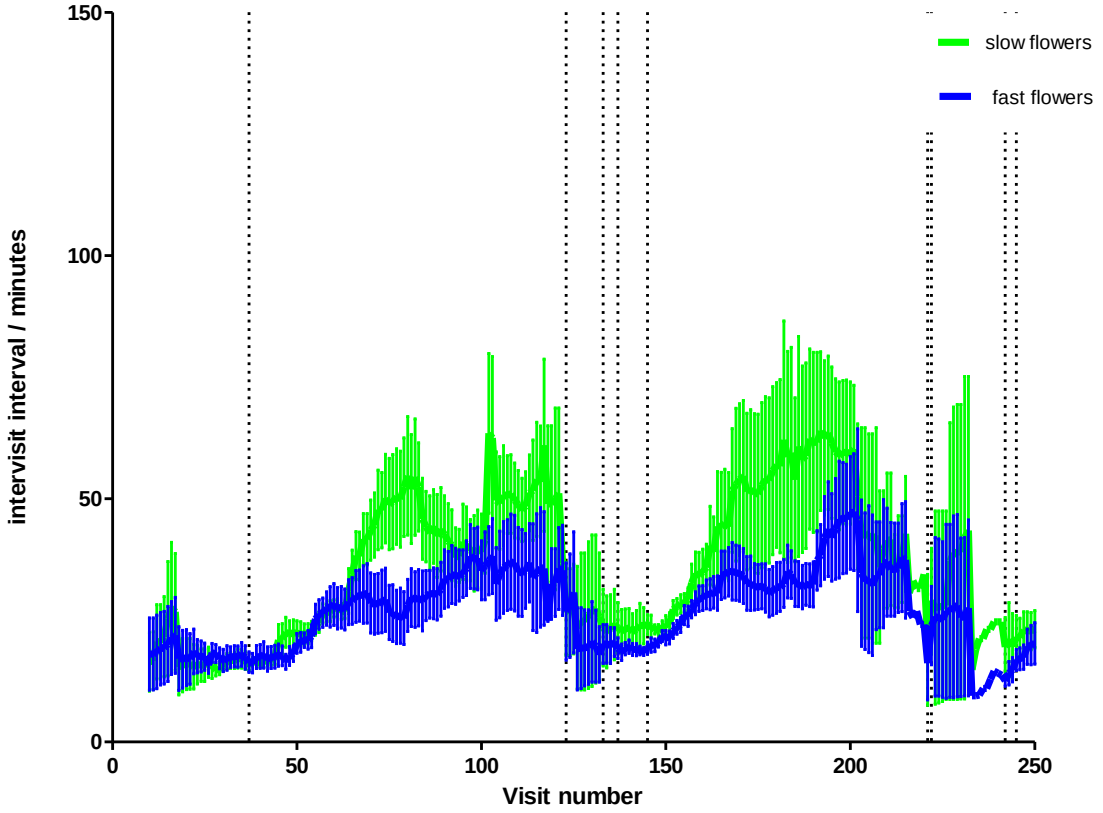

Figure 3. *Return times (inter-visit intervals; mins) to slow and fast flowers as a function of visit number in Experiment 1, plotted as a running average across blocks of ten visits. The green line denotes slow flowers and the blue line denotes fast flowers. Dotted lines represent the points at which training stopped and restarted for any one of the five birds, either due to the start of a new days training or due to rain. Averages were not calculated across adjacent training sessions. Error bars: ± S.E.M.*

Figure 3 reveals that, although there was generally an increase in inter-visit interval across training, there were also occasions when birds returned to flowers more frequently and with less variability. These seemed to coincide with periods where training was restarted after a break, caused by the end of the day or by rain (both types of event are marked on Figure 3 with dotted lines). To investigate the causes of this pattern further, and to determine whether the changes in revisit rates to flowers across training found in the preceding model were purely caused by the distribution of these periods of fast return time and low variance, we constructed a second model, modelling log inter-visit interval as a function of the number of visits made by the bird since the last time the experiment had been restarted for any reason, flower type, day of training, and time of day (morning or afternoon). We included interactions between flower type, time of day, and visit number since last restart, between flower type, day of training and visit number since last restart, the interaction between time of day and day, and all subsequent second-order interactions. Only two of the five birds progressed to a fourth day of training, resulting in the collection of only 111 data-points for Day 4, in comparison to the 430-535 each for Days 1-3. As this analysis is rather data heavy, we judged that we had insufficient data to support an analysis comparing Day 4 to the rest of training, hence the data from Day 4 have been excluded.

There was a significant interaction between flower type, visit, and time of day, (Model AIC = 909.50, model DF = 1476, $T = 2.46$, $P = 0.014$), demonstrating that time of day did have an effect on revisit rates, but that this effect was moderated by flower type and number of visits since last restart. This interaction is illustrated in Figure 4. There was also a significant interaction between day, visit, and type. In particular, the third day differed significantly from the first day ($T = -2.45$, $P = 0.014$) although the

second day did not (T = - 0.328, P = 0. 743). Figure 5 shows return times to slow and fast flowers across training as a function of number of visits made by a bird since the beginning of the training session for Days 1 and 2 compared to Day 3. Inspection of this graph suggests that birds returned to slow flowers less often on Day 3 than on Days 1 and 2.

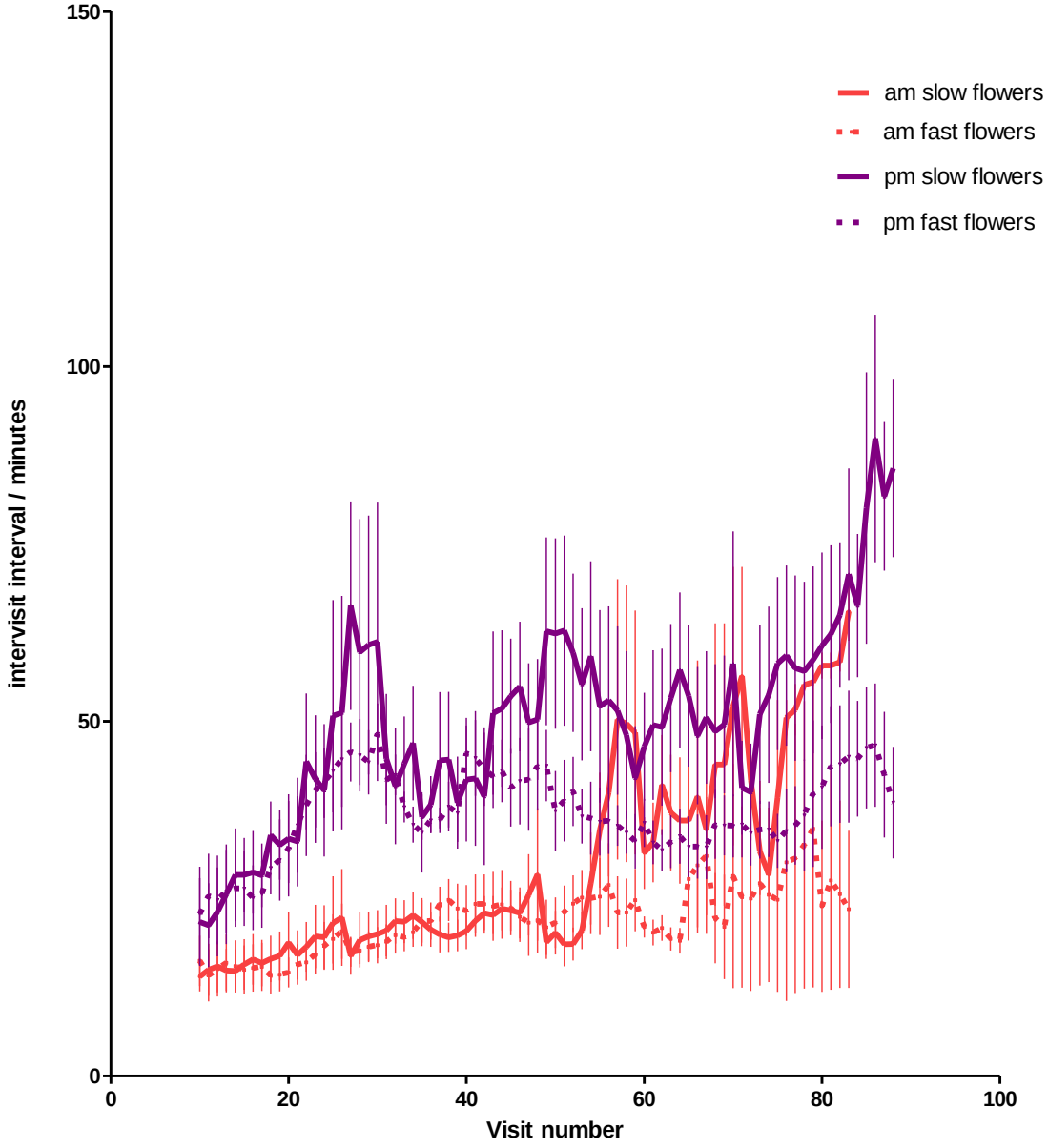

Figure 4. *Return times (inter-visit intervals; mins) to slow and fast flowers in Experiment 1 as a function of number of visits since a training session commenced. The red lines represent return times in the morning and the purple lines represent return times in the afternoon. The solid lines represent return times to slow flowers and dotted lines represent return times to fast flowers. The data are running averages over ten visits, across birds, and across all sessions and days. Error bars: ± S.E.M*

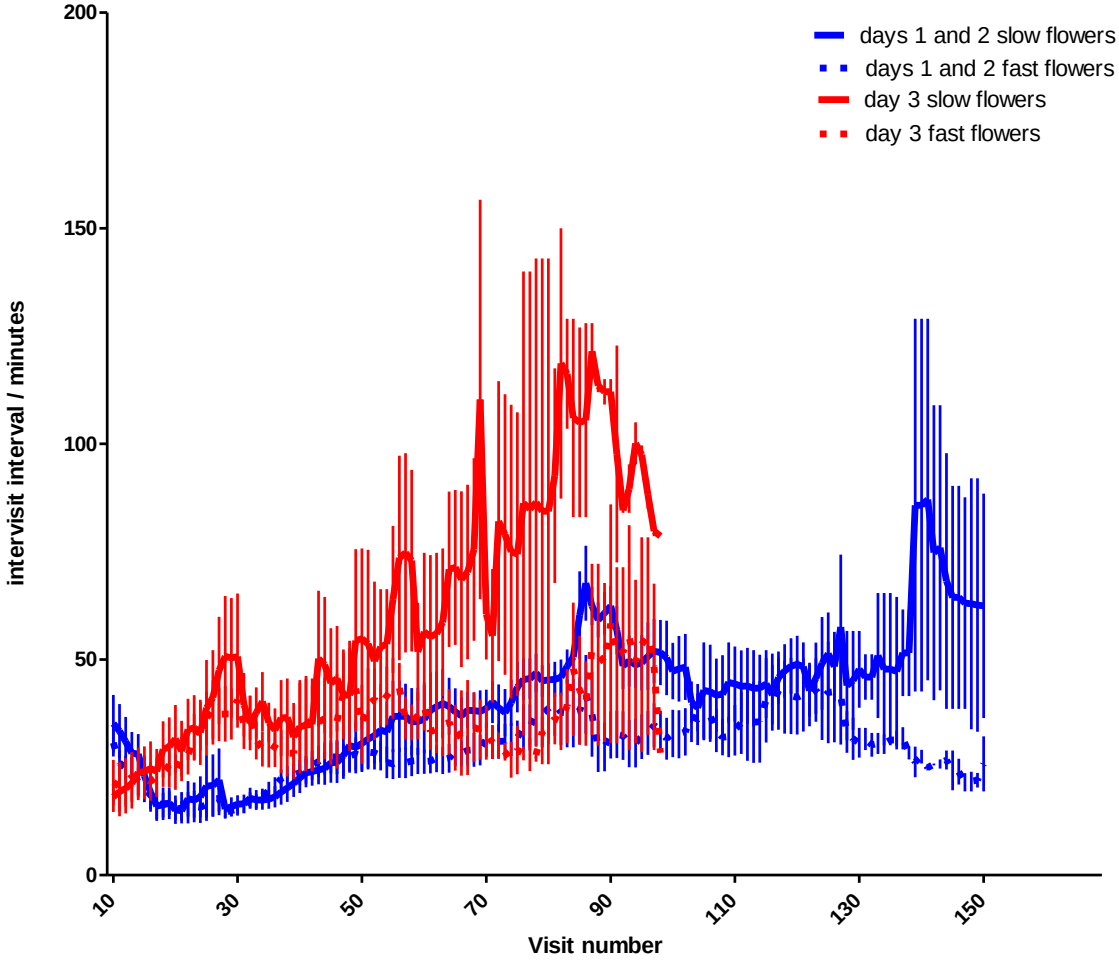

Figure 5*: Return times (intervisit intervals; mins) to slow and fast flowers in Experiment 1 as a function of number of visits since a training session commenced. The blue lines represent return times from Days 1 and 2 and the red lines represent return times from Day 3. The solid lines represent return times to slow flowers and dotted lines represent return times to fast flowers. The data are running averages over ten visits, across birds, and across all sessions within the relevant days (Days 1 and 2 for the black lines, Day 3 for the grey lines). Error bars: ± S.E.M.*

Experiment 2

To compare birds' choices of low and high volume flowers across training we constructed a binomial mixed effects model, modelling flower type as a function of visit number, with a random effect of bird on the model intercept and on visit. Birds did not choose high volume flowers more often than low volume flowers across training

(Model AIC = 1261, N = 904, model intercept 0.059 ± 0.119, Z = 0.501, P = 0.616) and training did not change their flower choice (Z = 0.342, P = 0.733, see figure 6). Birds' PRPs to rewarded and unrewarded flowers were not amenable to modelling as in Experiment 1.

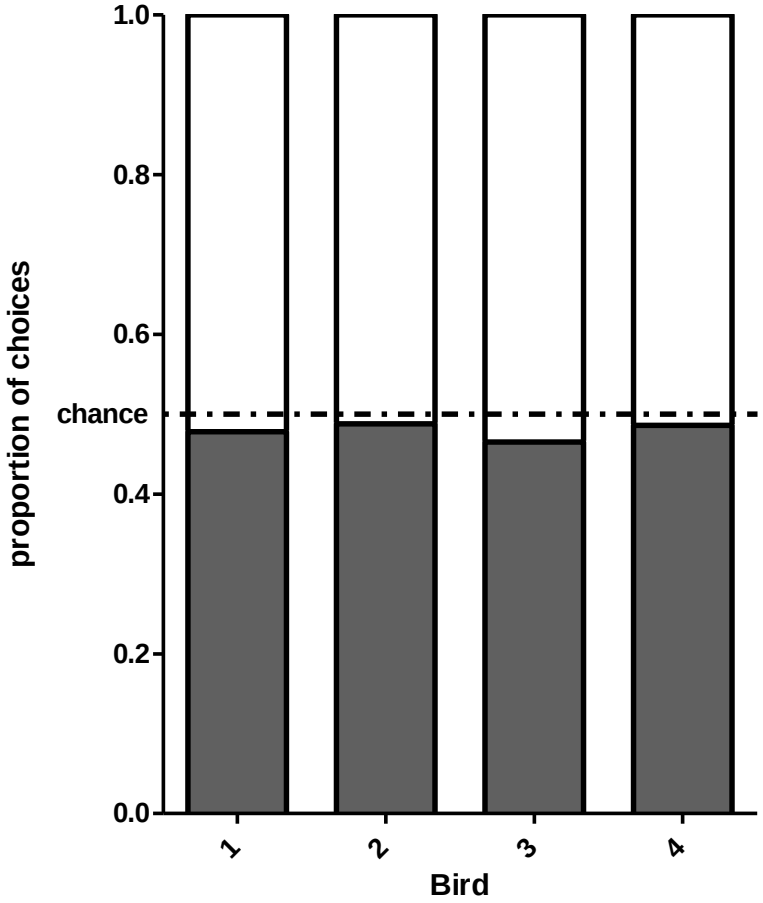

***Figure 6**: Proportion of choices to high and low volume flowers in Experiment 2. The grey bars denote low volume (5 µl) flowers and the open bars denote high volume (10 µl) flowers.*

Discussion

In Experiment 1, hummingbirds visited flowers with fast refill rates more often than they visited those with slow refill rates (Figures 2, 3, 4 and 5). They were also able to detect the difference in rates very quickly, shown by the fact that their choices between fast and slow flowers are not at chance from the beginning of training. This study demonstrates that birds can not only learn about resources that vary in their reward state with time in an all-or-nothing manner with respect to time, but that they can also learn about resources that change gradually.

Previously, the strongest evidence that hummingbirds could detect changes in the rate at which flowers refill came from the apparent matching by long-tailed hermit hummingbird *Phaethornis superciliosus* of the decline across the day in the rate their flowers supplied sucrose by visiting the flowers less often as the day progressed (Garrison and Gass, 1999). However, as the long-tailed hermit hummingbirds also decreased their feeding rate across the day when fed under *ad libitum* conditions (Gass and Garrison, 1999), it was unclear whether the birds' diurnal pattern of feeding caused an apparent, rather than real, matching of the gradual pattern of flower refilling. Time of day did affect feeding rate of our birds too: Figure 4 shows that birds visited flowers more slowly in the afternoon than they did in the morning, consistent with established patterns of hummingbird foraging (Gass and Garrison, 1999, Stiles, 1995, Stiles and Wolf, 1979), although this effect was moderated by flower type and how long it had been since the experiment was last restarted. Our birds also fed much more frequently at the beginning of each training session than they did near the end of a session (Figure 3), which we interpret as a response to the accumulation of sucrose in flowers as training progressed. Furthermore, in our experiment, by keeping refill rates constant

throughout the day, we could demonstrate that birds were sensitive to different refill schedules in a way that was not purely controlled by changes in feeding rates within days: by Day 3, birds returned less often to slow flowers than to fast flowers (Figure 5).

Although the birds were able to detect a difference in slow and fast refill rates, their responses birds did not conform to our prediction that they should visit more rewarding flowers twice as often as less rewarding flowers, in order to forage optimally (a strong form of the Matching Law, which has been found in its more general form in numerous species and circumstances, reviewed in Williams, 1988). Whilst birds did differentiate between the two flower types, they visited the slow-refilling flowers on average on 42% of their visits rather than on 33% of visits. The duration between visits to the slow-refilling flowers should also have been twice as long as that to the fast-refilling flowers, which it was not (Figure 3). Our birds did not therefore conform to the strong form of the matching law. It is not clear why the birds' visits did not conform to these two predictions. Unless there was some systematic error in our sucrose delivery procedure that tended to make the two flower types more similar, the birds may have not matched refill rates either due to some cognitive limitation or because our prediction of what constitutes optimal feeding from these flowers did not take into account all of the factors directing birds' behaviour (Houston et al., 2007).

One cognitive mechanism that might account for the birds visiting the slow flowers more often than we expected is that many animals find it difficult to withhold from taking a reward in the present so as to receive a greater reward in the future (Dufour et al., 2007, Paxton and Hampton, 2009). Potentially, hummingbirds do not allow sucrose to accumulate in flowers because, as flowers generally contain at least some reward (except in the five minutes following the last feeding bout), giving flowers

more time to refill requires birds to forego a reward in the present. We think this is unlikely to be the case here, as hummingbirds feeding from flowers that refilled over longer intervals, and without sucrose accumulating across those intervals, also did not match their visit rate to reward delivery rate (Henderson et al., 2006, Marshall et al., 2012).

The early returns to slow flowers might also be due to imprecision in the birds' perception of refill rates. While this is explanation is plausible, across birds, the rate of visiting slow flowers as opposed to faster ones was such that each hummingbird visited slow-refilling flowers at a rate that was more similar to the visit rates of the other birds than was any single bird's percentage use of slow flowers to the predicted 33% (the five birds tested revisited slow flowers at 40%, 40%, 42%, 44% and 44% of the visits). It is also possible that the difference between the predicted and observed use of flowers was caused by the small number of birds tested. However, the consistency between birds in our opinion makes this unlikely, although not infeasible.

The final possible explanation for birds' behaviour is that they are not foraging with a view to solely maximising their energy intake rate. For example, it might be that the birds were foraging for information (Inglis et al., 1997), an activity that would act to make revisit rates to the two flower types here more similar. Hummingbirds are also thought to defend flowers via foraging, making their floral resources unprofitable for other nectarivores by keeping their reward levels constantly low: defence by exploitation (Gill, 1988, Paton and Carpenter, 1984). If birds revisit flowers more often than they would otherwise, in order to keep the sucrose available to intruders low, the costs and benefits of this behaviour may differ between fast and slow refilling flowers: in particular, the pilfering of a high volume of sucrose from a slow-refilling flower

would represent a more costly loss than the same volume taken from a fast-refilling flower, due to the increased time slow flowers take to refill. This difference may then cause the departure from matching seen here.

Given that the birds appeared to be able to distinguish the difference in refill rates between the two groups of flowers in Experiment 1, the results of Experiment 2 were surprising. Birds did not differentiate between flowers containing 10µl and 20µl, and they did not become more discerning with training. This may have been because they were unable to detect the difference between these two volumes. We think this is unlikely as rufous hummingbirds can discriminate between 10 µl and 20µl, strongly preferring wells containing 20µl over wells containing 10µl (Morgan et al., 2012). It seems more likely that the apparent lack of discrimination between the flower types by the birds in Experiment 2 was due to birds visiting a large number of flowers per bout: on average birds visited six flowers per bout and they visited all eight flowers on 24% of visits. This high visitation rate is likely to have made differentiating between flower types both more difficult to detect by the experimenter and less important for the birds. Furthermore, the birds had much less experience of this array than the birds had in Experiment 1 so the comparison of the birds' abilities across the two experiments is not necessarily appropriate.

In sum, hummingbirds can detect and respond to gradual changes in the rate at which flowers refill, but they do not do so in a way that is predicted by optimal foraging. While this might be due to a perceptual or cognitive constraint, it is also plausible that these highly territorial birds return earlier to flowers so as to reduce the standing crop that might be available to intruders.

5: Deconstructing memory: What, where and when

Introduction

Episodic memory is the system by which humans recall details of their past experiences and is distinguished from semantic memory by the way in which information is remembered. Whilst semantic memory covers "known memories", such as remembering that the battle of Hastings took place in 1066 for example, an episodic memory is made up of the actual experience of an event (Tulving, 1993). Tulving (2002) has suggested that episodic memory relies on such faculties as autonoetic consciousness and a sense of subjective time, which would render episodic memory either a uniquely human ability or at least accessible currently only in humans.

In an attempt to counter this inaccessibility of episodic memory in animals, researchers have redefined the problem with the aim of capturing the functional, but not necessarily the mechanistic, aspects of this memory domain, under the banner of "episodic-like" memory. This definition reflects the interest in this work in the content rather than the form of memory. Episodic-like memory has been defined as the ability to integrate the what, the where and the when aspects of a past event and to use that memory flexibly to guide behaviour (Clayton et al., 2003a). As a result of using this definition, the ability to act on these three components of a past event simultaneously has now been demonstrated in a range of species (including scrub jays, *Aphelocoma coerulescens*: Clayton and Dickinson, 1998, chickadees, *Poecile atricapillus*: Feeney et al., 2009, meadow voles, *Microtus pennsylvanicus*: Ferkin et al., 2008, and rats *Rattus norvegicus*: Babb and Crystal, 2006).

This use of a functional rather than a mechanistic definition has, however, led to considerable debate regarding the extent to which episodic-like memory resembles

episodic memory (see: Clayton et al., 2003b,Suddendorf and Busby, 2003b, Suddendorf and Busby, 2003a). The central tenet of episodic memory, the experience of the individual, is currently untestable and seems likely to remain so for the foreseeable future. However, we contend that there are other features of episodic memory that are amenable to investigation within an episodic-like framework. For example, episodic memory in humans is regarded as constructive (Conway and Pleydell-Pearce, 2000, Schacter and Addis, 2007, Schacter et al., 1998): memory does not store events whole like a tape recorder, but rather via a system in which the elements of a memory are stored separately and recombined to create the event as it is recollected. In contrast, episodic-like memory investigations typically require an animal to demonstrate that it can remember the three memory components together and to have failed if any of the elements is absent. If, however, the same mechanisms underpin episodic-like memory and episodic memory, the components of memory of the former should also be dissociable and recombined at the point of recall. This recombination may on occasion be imperfect or incomplete, as is often the case for human memory. By looking at the separate elements of episodic-like memory: what, where and when, and how these three are combined to direct behaviour, we may therefore be able to make some progress in the debate over how closely episodic-like resembles episodic memory.

Here we attempted to deconstruct the ability of hummingbirds to remember the what, the where and the when of the flowers on which they were foraging. These flowers were either rewarded, or not, according to an experimentally-determined temporal rule. In the first experiment the temporal component could be remembered either by the sequence of rewarded flowers or by the time of day. In the second experiment we investigated which of these types of time the birds used. In both

experiments, birds were trained to feed from a single rewarded flower of eight artificial flowers, arranged in two square arrays of four flowers. A single flower from one array of four was rewarded in the morning and a different coloured flower in the other array in the afternoon. The four flowers in each of the two arrays differed in colour but the colours and relative spatial relationships among the flowers were the same for both arrays. In this way, a bird's flower choices could be classified as correct (selecting the rewarded flower) or incorrect with regard to what (colour), where (array) or when (time of day or order). This allowed us to examine both the nature of the errors birds made as well as whether the birds could remember all three components of a memory. In Experiment 1, we predicted that, whilst birds should be able to learn to use what, where and when information to guide their behaviour successfully, these components should be dissociable, manifested by some aspects being easier to learn than others. In particular, we predicted birds would find the "when" component of the task hardest to integrate, as demonstrating memory for "when" has frequently been a stumbling block in episodic-like memory research.

In Experiment 2, Birds were trained to the same task, then underwent three novel tests, one earlier than the morning training session, one midway between the morning and afternoon training sessions, and one later than the afternoon session. Depending on which of the time cues birds used, we predicted that the birds would choose different flowers in these three tests: when presented with the array earlier than the first trained time, birds should visit the rewarded flower from the morning array, irrespective of whether they are relying on order or time of day, as this is both the first presentation of the day, and occurring in the morning. At midday, if birds use time of day information, they should visit the two rewarded flowers equally, as this test occurs

midway between the morning and afternoon times, whereas if they use sequence information they should visit the flower rewarded in the afternoon, as they will have already experienced feeding from the morning rewarded flower. In tests after the afternoon training session, birds using time of day information should also visit the flower rewarded in the afternoon, whereas if birds use sequential information to predict the reward location they should visit the morning flower, as the most recently visited flower would have been the afternoon flower.

Methods

Subjects

The subjects in these experiments were 18 free-living male rufous hummingbirds defending feeding territories along the Westcastle Valley, in the Eastern range of the Rocky Mountains (49º 21'N, 114º 25'W), Alberta, Canada (12 in Experiment 1, and 6 in Experiment 2). Each territory was centred on a single hummingbird feeder, containing 14% sucrose solution. Birds were marked on their breast feathers with a small amount of non-toxic ink, to allow individuals to be identified. Observations were conducted between 0730 and 1930 (Mountain Standard Time). Experiment 1 was conducted in June-July 2005, and June 2006, and Experiment 2 from June-July 2008. All work was carried out under permit from Environment Canada and Alberta Sustainable Resource Development with the ethical approval of the University of Lethbridge Animal Welfare Committee.

Pre-training

All birds were initially trained to feed from artificial flowers consisting of a 6cm diameter cardboard circle, coloured either red, blue, purple or pink, with a syringe cap in the centre, forming the "nectar well", which was filled with roughly 600ul of 20% sucrose. Each flower was mounted on a small cork glued to a 60 cm high wooden stake. This flower was gradually moved to the site of the birds' first training array. Once the bird had fed at least four times from this flower, the next stage of training began.

Experimental Training

Birds first received training for the "Morning" session. Each bird was presented with four flowers, coloured blue, red, pink or purple and arranged in a rough 60 cm by 60 cm square. The flower of the training colour contained sucrose while the remaining three were filled with water, which the birds find unpalatable. The bird was allowed to visit this array until he had fed from the sucrose-filled flower six times, after which time the flowers were removed and the bird's feeder replaced. This completed the training for the 'Morning' session. The 'Afternoon' training began at least four hours later or, in cases of bad weather, in the afternoon of the following day. A flower of a different colour to that rewarded during morning training was placed below the bird's feeder, then moved towards the site of the Afternoon array, which was located roughly 10 m away from the location of the Morning array. The bird was then presented with a second array of four flowers in this new location, with the flower of the colour he had just fed from rewarded, and the flower colour from the Morning array, and the remaining two flower colours, containing water. Again, the bird was allowed to feed

from the rewarded flower 6 times. The morning following the completion of both the Morning and the Afternoon training sessions, the experiment proper began.

Experiment 1

Twelve birds were used in this experiment. In the morning (at any time between 0730 and 1100, depending on the time of the Morning pretraining a bird had received), a bird's feeder was removed and he was presented with both the Morning and Afternoon arrays of flowers simultaneously, arranged as they had been during training. The only flower of the eight to contain sucrose was the one that had been rewarded in the previous morning's training. All the remaining seven flowers contained water. The bird was allowed to visit any of the flowers until he had made six visits to the sucrose-filled flower, which was refilled after each visit, at which point both arrays were removed and the feeder replaced. At the time of Afternoon pre-training, both arrays were returned but the rewarded flower was now that rewarded during the Afternoon pretraining, and the Morning rewarded flower now contained water. Again, the bird was allowed to visit flowers until he had visited the sucrose-filled flower six times. His feeder was then returned for the remainder of the day. The number of days males were tested varied from 3-9 (median = 7). All visits to all flowers were recorded at both Morning and Afternoon sessions. A schematic of the experimental layout is shown in Figure 1.

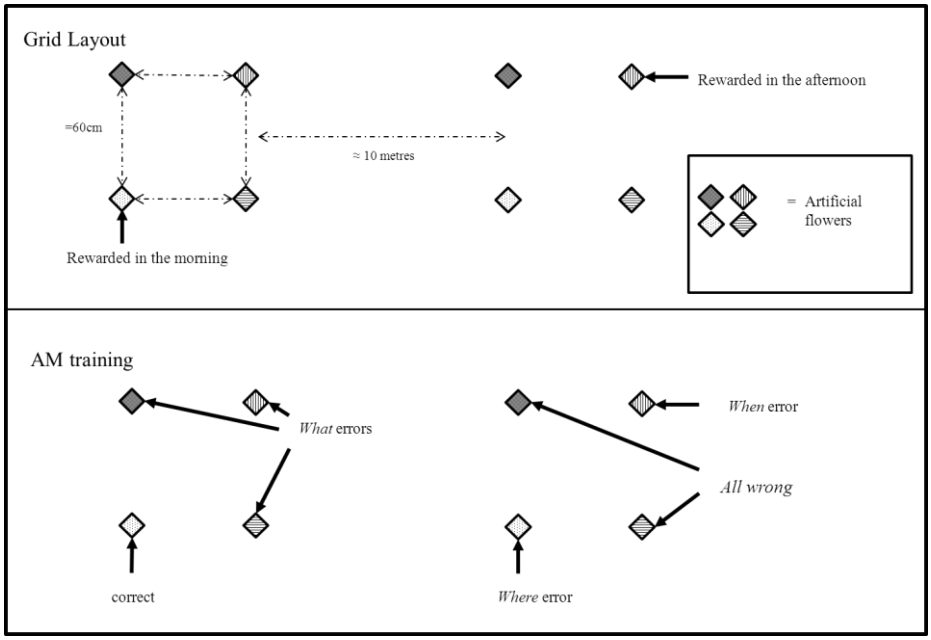

Figure 1. *Schematic showing sample grid layout for Experiment 1, and flower categorizations for morning trials.*

Experiment 2

Six birds were tested in this experiment. As for experiment 1, birds were trained with two arrays of four flowers twice a day, with only one of the eight being rewarded at a given presentation, and that flower being consistent with the flowers rewarded at that time of day during pre-training. As before, a bird was allowed to feed from the rewarded flower six times on each session, then the feeder returned and the array removed. Training occurred over six days: one day of pretraining and five days of training to the full array. Following training, birds received three tests: an Early test, two hours before morning training, a Midday test, in-between training sessions and a Late test, two hours after training was completed. Birds were allowed to make one visit to the array at each of these tests, after which all flowers were removed and the feeder replaced All flowers were empty during these tests. During testing, birds continued to experience Morning and Afternoon training at the usual times, and birds only

experienced one test session per day. The order of the tests was pseudo-randomised between birds.

Results

Experiment 1

We were firstly interested in birds' first choices on approaching the array. Therefore, we categorised each of the first visits made by each male on each training session according to the type of decision made (see Figure 1): a *Correct* decision was to select the rewarded flower (chance = 0.125). Relative to this choice, all other decisions were 'errors', of which there were four kinds: "*What"* errors, where the bird chose the correct array at the correct time but a flower of the wrong colour (chance = 0.375); "*Where"* errors, where the bird chose a flower of the correct colour but in the wrong array (chance = 0.125); "*When"* errors where the bird chose the flower of the correct colour and in the correct array, but that was the flower that was rewarded at the alternative time (chance = 0.125); *"Fail"* errors where the bird chose a flower of the wrong colour, in the wrong place at the wrong time (chance = 0.250).

We had predicted that birds would correctly recall "*What*", "*Where*", and "*When*" more often than expected by chance, but would find "*When*" the hardest of the three aspects to remember. To test this we firstly compared the proportion of correct choices males made with that expected by chance, using a one-sample T-test, after arcsine square root transforming the data. As expected, males made correct decisions significantly more often than expected (one-sample, two-tailed T-test: $N = 12$, $T = 6.533$, $P < 0.001$, $d = 1.89$; Figure 2).

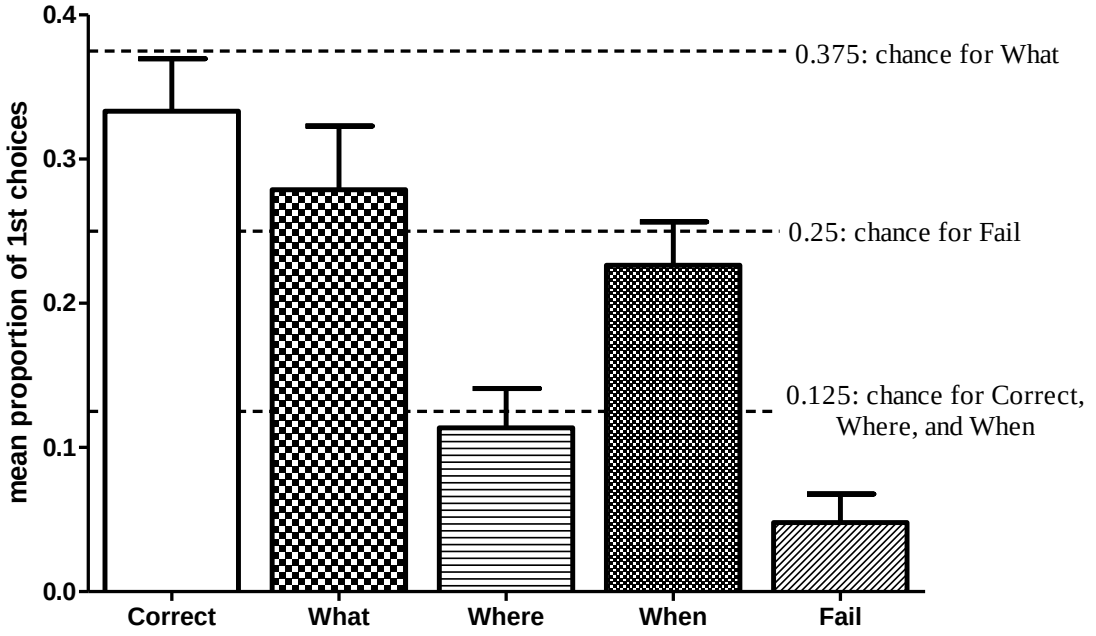

Figure 2. *Proportion of first choices of each type between birds during training in Experiment 1. The solid line at 0.125 represents chance for when, where, and correct choices . The dashed line at 0.375 represents chance for what choices. The dotted line at 0.25 represents chance for fail choices . Birds made correct choices significantly more often than chance (One-sampled, two-tailed T-test, n=12, T=7.284, p<0.001). Error bars represent ± SE.*

We then looked at the mistakes birds made. These could be "*What", "Where", "When"*, or "*Fail*" errors. We recalculated the probabilities of birds making each of these types of errors, conditional on them having failed to make a correct choice. We compared the arcsine transformed square root transformed proportion of "*When*" errors made to the arcsine square root transform of this new probability (0.143, or 1 in 7). As expected, birds made more "*When*" errors than expected (one-sample two-tailed T-test: $N = 12$, $T = 4.885$, $d = 1.41$, $P < 0.001$). Because of the nested structure of these data it is difficult to analyse the proportions of other errors made. However, it appears that

"*What*" and "*Where*" errors occurred at around chance levels, whilst "*Fail*" errors were very rare (see Figure 3).

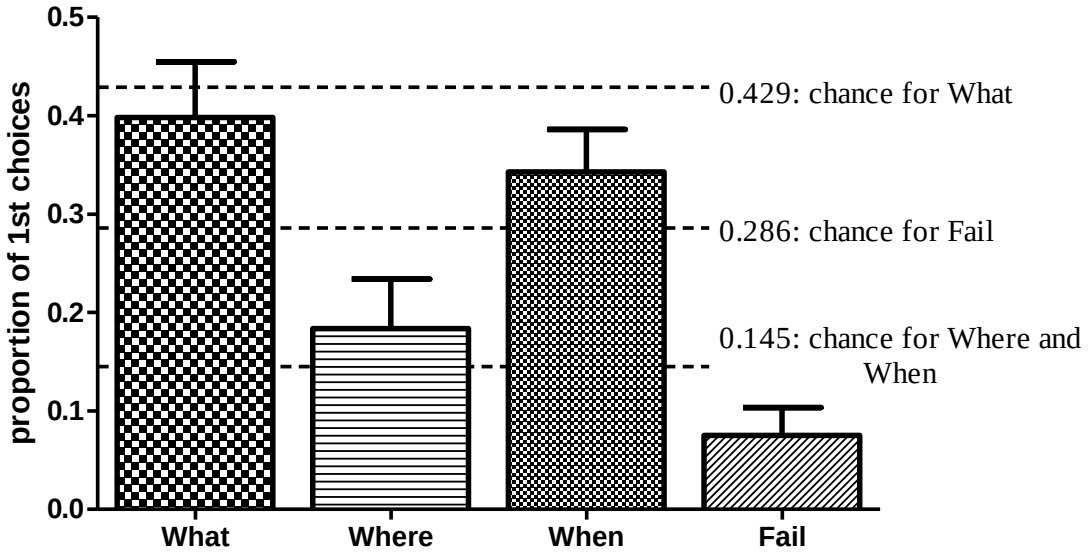

***Figure 3**: Proportion of first choice errors of each type between birds during training in Experiment 1. The line at 0.145 represents chance for when and where errors. The line at 0.429 represents chance for what errors. The line at 0.286 represents chance for fail errors. Birds made When errors significantly more often than expected by chance (One sampled two-tailed T-test, n=12, T=5.083, p<0.001). Error bars represent ± SEM.*

There are two different aspects of birds' mistakes we can examine in this experiment: the kinds of mistake made, and how the birds went about correcting them. To determine whether some mistakes were easier to correct than others we constructed a partition tree in R, using the package rpart, using the data for each bird's errors and subsequent choices. This type of analysis produces a classification tree, similar in form to a species identification key, which can be used to identify a hierarchy of factors determining whether a mistake is likely to be corrected or not. The data from each trial were truncated at the point each bird successfully located the reward, up to the first four

visits within a training trial (birds failed to find the rewarded flower within the first four visits on only 19 of 115 total trials). We coded each visit by trial number (a measure of experience), visit number (a measure of how many attempts they had already taken), the type of error made on the last visit, and the bird in question. The response variable was whether the bird made a correct choice or an error on a given visit. This analysis therefore allows us to determine whether the type of mistake last made, level of training or number of mistakes already made determines whether or not a mistake is corrected on the next visit, how these factors may interact with each other, and whether birds are idiosyncratic. The resulting pruned tree is shown in Figure 4.

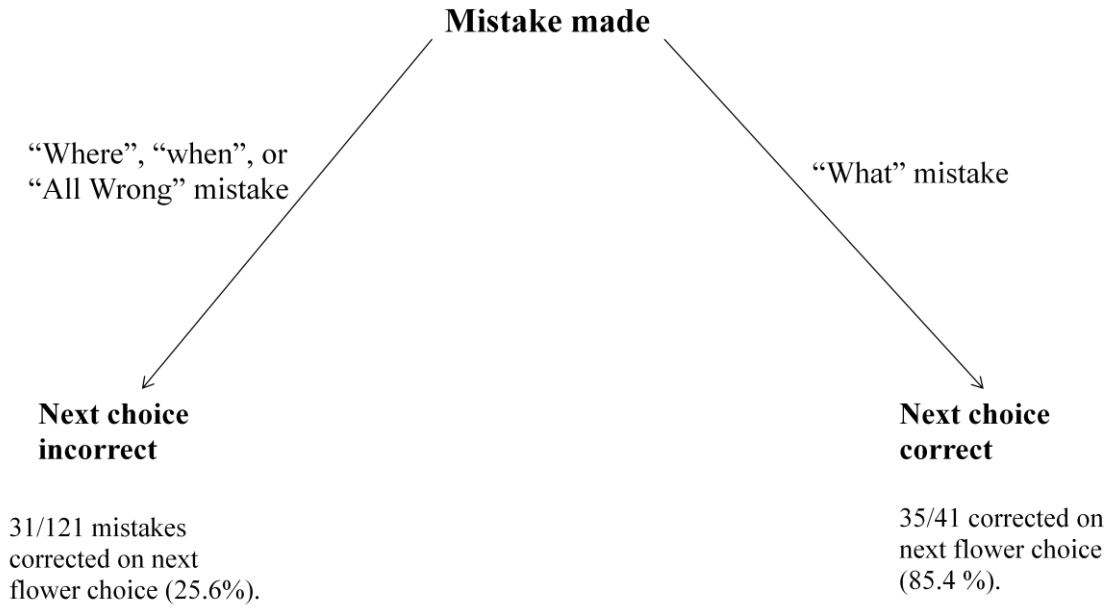

Figure 4. *Partition tree of the factors determining whether or not an error will be corrected on a birds' next choice. The only important factor in determining whether or not a mistake will be corrected is the type of error made on the last trial, with "what" errors being corrected more easily than any other sort.*

The only factor in this tree is the type of error made in the last trial, with birds being more likely to correct a "*What*" error than a "*Where*", a "*When*" or a "*Fail*" error. That this is the only factor remaining in the tree means that the type of error made at the last choice has a greater predictive power in whether an error will be corrected than the length of training or the number of attempts he has taken on a trial Whilst 85% of "*What*" errors were corrected on a bird's next flower choice, only 26% of errors of other types were**.**

Experiment 2

We looked only at the first choice of each bird in the three tests. In the Early tests, all six birds chose the flower that contained the reward in the 'Afternoon' array. In the Midday tests, there was less of a consensus among the birds in flower choice, although they did not choose randomly among the eight flowers. Four birds chose the flower that was rewarded in the Morning array while two birds chose the Afternoon flower. There were no choices to any of the six flowers that never contained reward. In the Late tests, five of the six birds chose the Morning flower, whilst the 6^{th} bird chose a flower that was not consistent with either time of day (see Figure 5).

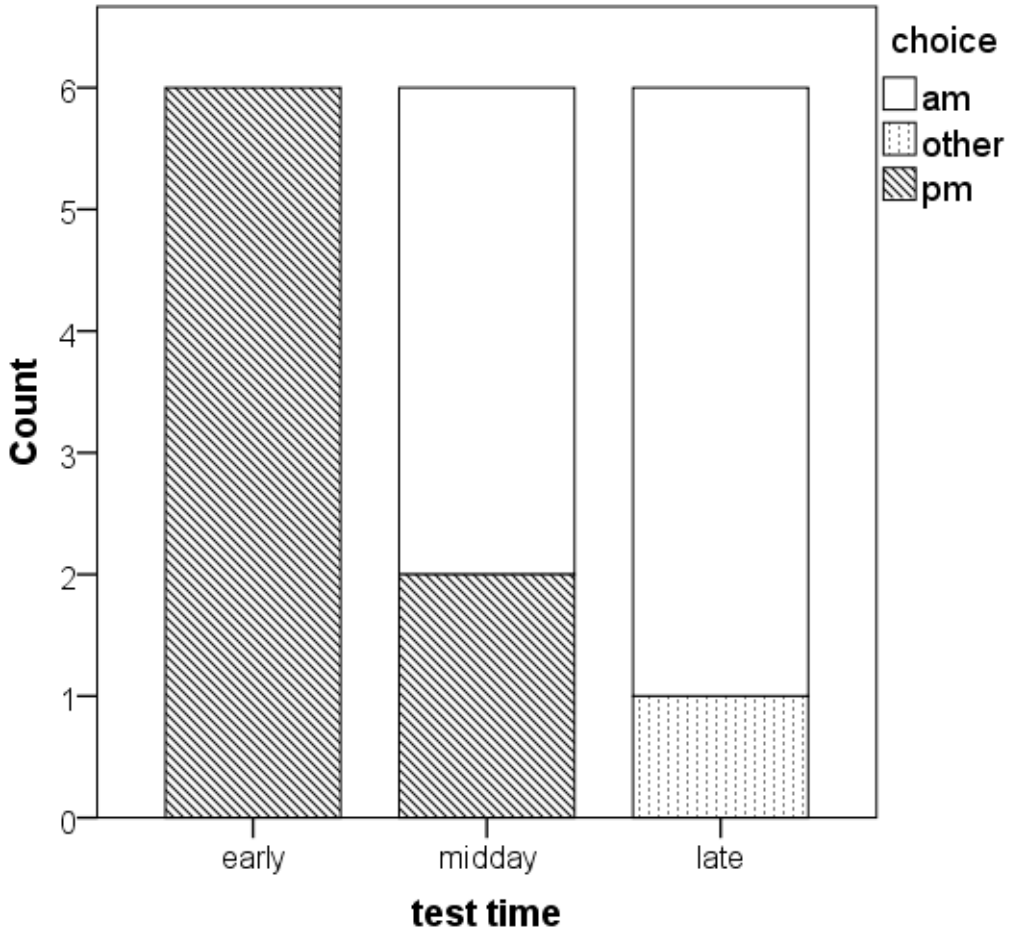

Figure 5*. The number of birds that made their first choice to the Morning and Afternoon flowers at the Early, Midday, and Late tests. Striped portions of bars represent choices of the pm rewarded flower, clear bars represent choices of the am rewarded flower, and dotted bars represent choices of unrewarded flowers.*

As the aim in these tests was to compare which of the two flowers (Morning or Afternoon) birds expected to contain the reward at untrained times of day, we modelled this test as a binary choice, where birds could either choose to visit the Morning or the Afternoon flower. If birds did not use any form of time rule but had just learned which two flowers were rewarded, then their visits to these two types of flower should be equal at all tests. We tested birds' performance against this null hypothesis using a binomial test.

In Early tests, the birds' chose the flower rewarded in the Afternoon significantly more often than expected by chance (two-tailed test binomial test: $N = 6$, test-proportion = 0.5, exact $P = 0.031$; Figure 5). In Midday tests, the birds' performance did not differ significantly from chance (two-tailed binomial test: $N = 6$, test-proportion = 0.5, exact $P = 0.688$). In the Late tests, birds showed a near-significant preference for the flower rewarded in the morning during training (two-tailed test binomial test: $N = 5$, test-proportion = 0.5, exact $P = 0.063$).

As a further indication of which time system birds were using, we also looked at their behaviour at the next normal feeding exposure following an unexpected test. If birds use a time of day system, they should still choose the correct, rewarded flower, as the test should not interfere with birds' knowledge that this flower is rewarded at this time of day. If birds are using a sequential rule to predict food availability, their behaviour is less easily predicted: if the unrewarded single visit made by a bird during a test is perceived by birds as a sequence element, they may alternate their choices on the next visit, leading them to chose the incorrect flower. However, as the test is unrewarded and short, whether birds would perceive it as part of the sequence (and which part if so) is not clear, hence predicting birds' choices if they are using a sequential rule is not straightforward. This analysis therefore has the potential to rule out exclusive use of time of day as a director of birds' behaviour, but not to prove that either system is being used exclusively. Birds' behaviour following Early and Midday tests was not significantly different from chance (two-tailed test binomial tests, Early test: $N = 6$, test-proportion = 0.5, exact $P = 0.687$; Midday test: $N = 5$, test-proportion = 0.5, exact $P = 1.00$).

Discussion

Rufous hummingbirds can remember the what, where and when aspects of rewarded flowers. Furthermore, as 33.3 ± 3.6% of first visits are to the correct flower it appears that these birds can remember all three pieces of information together. Birds' errors were not, however, random. In particular, birds were most likely to make errors regarding when a flower should be rewarded. This is consistent with much of the episodic-like memory literature, where the time component appears to be the most difficult for many animals to learn (Bird et al., 2003, Hampton et al., 2005). It appears, then, that, for these birds, what, where and when are stored as separate pieces of information in memory, as is thought to be the case for human episodic memory (Schachter et al 1998).

Although there did not seem to be any difference in how well "*What*" and "*Where*" were remembered, "*What*" mistakes were easily corrected. Here, "*What*" is signalled by flower colour, and previous research has suggested that, while hummingbirds can use colour to direct foraging when no other cue is available (Bateson et al., 2002, Bateson et al., 2003) colour information tends to be overshadowed by spatial information (Hurly and Healy, 1996, Hurly and Healy, 2002, Marshall et al., 2012). The ready correction of "*What*" mistakes would suggest that birds know the correct flower colour but preferentially use other information first. It is however worth noting that not all "*What"* errors in this experiment are equivalent: birds may either chose the flower colour rewarded at the alternative time (1 of 3 flowers), or a flower colour that has never been rewarded (2 of 3 flowers). It seems there is a difference in how readily birds make these two types of mistakes; most of the birds' *What*" errors in

training are to the colour rewarded at the other time (33 of 36 initial "*What"* mistakes), rather than to one of the two unrewarded colours. Given that most of these "*What"* errors are to a single flower, the proportion of "*What"* errors relative to chance are actually very high (see Figs 2 and 3). It seems likely that birds may have learned which flower colours are rewarded and which are not but their errors stem from failing to integrate this information with "*Where*" and "*When*". "*What"* errors are therefore most easily corrected as a bird knows the reward is in one of two flowers, and having ruled out the alternative can correct his decision on the next choice. It is also relevant to note that "*What*" here does also have a spatial component (that is, the flower's position in the array), so may also be thought of as a type of "*Where*" on a smaller spatial scale.

Based on the difficulty that animals typically have had in remembering the '*When*' component in the episodic-like data experiments to date, we predicted that the birds would find the '*When*' component of the task the most difficult to remember, which, indeed, they did. However, we may also have made this component of the task more difficult than the '*Where*' or '*What*' components because there is more than one kind of time the birds could have used to solve the task. Firstly, the birds could have learned which flower was rewarded by using the time of day at which flowers were presented. However, as the "Morning" flower was also always the first flower rewarded in the day and the "Afternoon" flower always the second, it is also possible that the birds learned which flower was correct by using a sequence or alternation rule.

The results of Experiment 2 suggest that birds did not exclusively use either of these systems, as, while the birds' choices of flowers in the tests were remarkably consistent and they fitted the predictions to some extent, this fit was only for part of each of the hypotheses: the results of the Midday test are consistent with a time of day

mechanism, the results of the Late test are consistent with the use of a sequential mechanism, while the outcome of the Early test, in which all six birds made the same flower choice, does not support either hypothesis.

However, the data can be explained with a single hypothesis: that birds' behaviour was produced by combining both time of day and order information, i.e. that the birds learned both sequence and time of day information and used both together to make their choices. The logic of this is as follows: for the Early test, birds detected that the array was not being presented at the Morning time so chose the alternative flower. Likewise for the Late test, birds detected that the array was not being presented at the Afternoon time so chose the alternative flower. The Midday results could also be explained this way because this time point was intermediate between both the two trained times of day and the two alternating sequence elements. We suggest, then, that the birds learned a sequence (or that the two flowers alternated) but that they also learned that the sequence was anchored to specific times of day. Certainly, there is evidence from rats that different types of time cue can be combined to guide behaviour (Pizzo and Crystal, 2002). This is also consistent with birds' behaviour following the unexpected Early and Midday tests, as at these times birds showed no significant preference for either rewarded flower, making 2/6 visits to the correct flower following the Early test, and 2/5 visits to the correct flower following the Midday test (1 bird visited a flower that was never rewarded). This suggests that birds were not exclusively using time of day, but cannot help us determine whether birds are exclusively using a sequence or integrating sequence and time of day, as how a short unrewarded trial would be perceived under a sequential rule is unclear. It is, however, important to note that the binomial tests used here are very intolerant to noise with small sample sizes (for

a sample size of 6 and a chance performance of 0.5, only a completely noise free result gives a significant result), so it is possible that birds do have a preference following these tests that we lack the power to detect, an argument that also applies to the Midday test results.

Under its original formulation, animals must remember all three of what, where and when simultaneously in order to show episodic-like memory (Clayton et al., 2003a). However, this all-or-nothing approach to memory is not in close accord with human episodic memories, which are often incomplete and which are thought to be recalled by utilising constructive processes, where an episode is reassembled as it is recalled (Schacter and Addis, 2007). Our suggestion that the what, where, and when components of episodic-like memory may likewise be separable receives some support from the work of DeVito and Eichenbaum (2010), who trained mice to a task in which what, where, and when memory could be measured separately to then compare the effect on task performance of hippocamal and prefrontal cortex lesions. They found that, although hippocampal lesions impaired performance on the overall task, lesions to the prefrontal cortex specifically impaired memory for the "where" element of the task, which appears to show that these components are separable anatomically as well as behaviourally.

The support our data offer to the idea that what, where and when are remembered in a constructive way by hummingbirds and possibly other animals suggests a number of ways to investigate the similarities (or not) between human episodic memory and animal episodic-like memory. In humans, the integrative nature of episodic memory leads to memory failures of a number of different sorts for numerous different reasons (beyond simple encoding failures), which may be amenable

to testing in animals. These include generalisation, where subjects incorrectly remember aspects of an event that did not occur due to their close relation to an event's actual contents. For example, subjects will frequently remember words that were not presented in a list if many thematically related words were presented, such as remembering *hospital* having studied the words *ambulance, doctor, operation, x-ray, ward* (Read, 1996, Roediger and McDermott, 1995). Another type of constructive memory error is blending, where two memories which share many features can be confused with each other, and combined to make novel (and inaccurate) memory (Nystrom and McClelland, 1992).

In sum, we contend that our study marks a significant advance step in the way we should consider the relationship between episodic and episodic-like memory. Specifically, by deconstructing episodic-like memories, we really might be able to determine the similarities (or not) between episodic and episodic-like memory.

6: Different types of time: Sequences and times of day

Introduction

In Chapter 5 hummingbirds were presented with a what, where, when task in which the location of a reward was predicted by two different temporal cues: order and time of day. After training, they were presented with three tests at untrained times of day: early in the morning, midway between the two trained times, and later than the second trained time, in order to determine what type of time cue birds had learned to use to solve the task. Whilst performances in those tests were consistent across birds, they were not exclusively consistent with either time rule: the result of the midday test suggested the use of a time of day rule, the afternoon test suggested a sequential or alternating rule, and the early test was consistent with neither hypothesis. In Chapter 5, we interpreted these results as arising from birds combining sequential and time of day information to guide behaviour. In order to test this explanation, however, we need to demonstrate that birds can learn both sequential and time of day information independently.

To this end, we presented 12 birds with a modified version of the task presented in Chapter 5. Birds were presented with an array of 12 artificial flowers, arranged into three groups of four, at three different times: for half of the birds these times corresponded to times of day, but occurred in a random order, for the other half of the birds these times corresponded to an order (1^{st}, 2^{nd}, or 3^{rd}), but occurred randomly with respect to time of day. Birds experienced six training sessions for each of these times, as in Chapter 5, after which they experienced an unrewarded test, to determine what they had learned. If the explanation we posited in Chapter 5 is correct, and those data do

reflect a combining by the birds of different timing cues, then the birds should be able to learn both these types of temporal rules.

Methods

This study was conducted in Westcastle Valley, Alberta, Canada, at 1400m elevation in the Eastern Rocky Mountains, 20 km southwest of Beaver Mines (49°292 N; 114°252 W). The subjects were 12 territory-holding, free-living, adult male rufous hummingbirds, feeding in territories established around artificial feeders containing 14% sucrose. Birds were marked on the breast with coloured ink at the beginning of the field season so that they could be distinguished without recapture. Trials were run from 7:00 to 21:00 hours Mountain Standard Time from May to July, 2010 and from May to June, 2011. All of the work was carried out under permit from Environment Canada and with the ethical approval of the University of St Andrews and the University of Lethbridge.

Initial Training

All birds were initially trained to feed from artificial flowers consisting of a 6 cm diameter orange cardboard circle, with a syringe cap in the centre, which formed the "nectar well". This contained approximately 600 µl of 25% sucrose, mounted on a 60 cm long wooden stake. This stage of training typically took an afternoon to complete. The following morning, this flower was placed below the feeder and the feeder removed, then moved in increments of roughly a metre to the site of the bird's first training array.

General Procedure

Each bird was presented with an array of 12 flowers, arranged into three groups of four flowers (a yellow, a blue, a pink and a purple flower) arranged in a square of side 1 metre. Each of these groups of four (hereafter referred to as mini-arrays) was at least two metres from any other and the positions occupied by each colour within an array of four were the same for each of the three sub-arrays. A typical layout is shown in Figure 1.

Each bird was presented with this array of 12 flowers repeatedly at three different times over a period of 6-9 days, depending on the group it was assigned to: Time of Day or Sequential. For the Time of Day birds, the three times, were specified by times of the day (e.g. 8am, 12pm and 4pm). For Sequential birds, the three times corresponded to the first, second or third presentation of the day. At each of these three times only one of the twelve flowers was rewarded. For each of the three time periods, the colour of the rewarded flower differed and that flower was located in a different mini-array. The remaining eleven flowers contained water. Six of the birds were assigned to the Time of Day Treatment group and six of the birds to the Sequential Treatment.

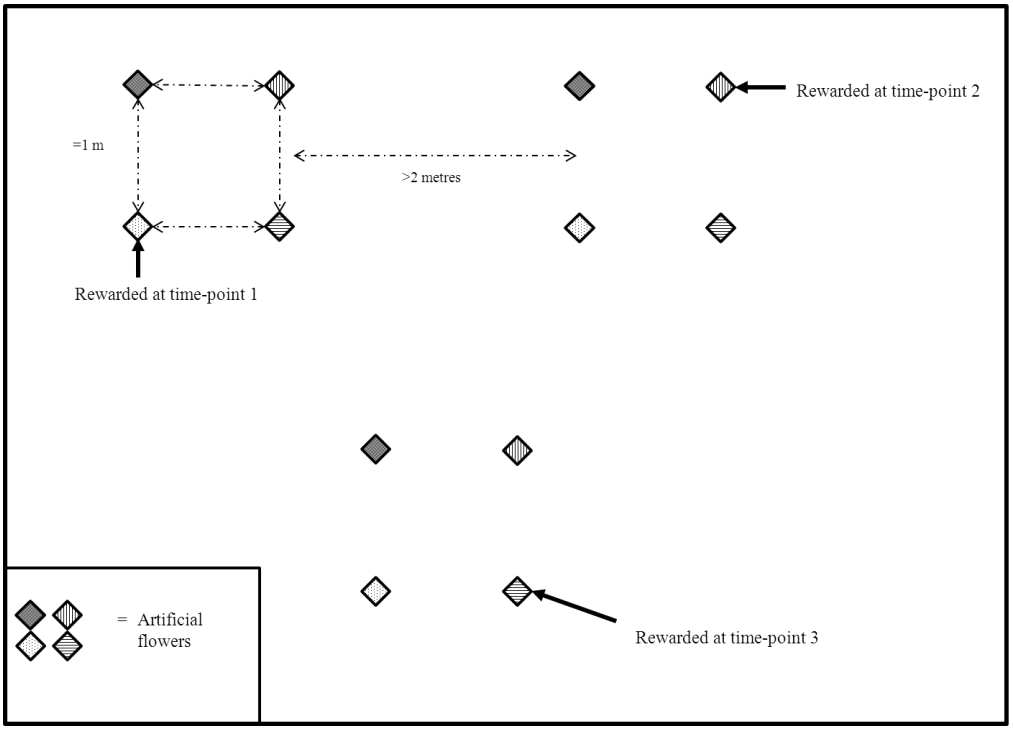

Figure 1*: Schematic of a typical experimental array for both Time of Day and Sequential birds. Arrays consisted of three sub-arrays of four differently coloured flowers, with colours occupying the same relative position within each sub-array. This array of 12 flowers was presented to birds at three different times, with a different coloured flower in a different sub-array rewarded at different times.*

Time of Day Treatment

Following the initial training to artificial flowers, the Time of Day birds were presented with a single flower (purple, yellow, pink or blue) containing at least 600µl of 25% sucrose. The first time the flower was presented was at the first time in the day of the designated times for that bird. For the six birds this varied from 8 am to 10 am. After the bird had fed from this flower, three other flowers (one of each of the remaining colours) were added to the rewarded flower so that they formed a 1m square array, comprising the first mini-array. These latter three flowers remained empty while the rewarded flower was refilled. Birds were then allowed to feed from this rewarded flower 5-6 times, over a period of up to about 75 minutes. A similar training procedure

then occurred at the sites of the second two mini-arrays at the appropriate time of day, with a different colour of flower being rewarded in each case, until the rewarded flowers had been fed from at least four times each. If a bird failed to feed from a rewarded flower at least four times within an hour and a quarter, the following day he was presented with a repeat of the pre-training for the relevant time.

The next day, Time of Day birds were exposed to the full array of 12 flowers, at two different times each day, consistent with the first, second or third times at which the mini-arrays had been presented during pre-training. At each time of day, the flower that had been rewarded at that time during pre-training was filled with 25% sucrose, whilst the other 11 contained water. Birds were allowed 60-75 min to explore this array and to feed from the rewarded flower up to six times per session. The time of each visit to the array, the flowers probed and the order in which they were visited were recorded for all sessions. Birds experienced nine days of training, corresponding to being trained to each time of day six times. So, for example, on the first day of training, a bird might see the array at time 1 and time 2 (8 am and 12 pm according to the earlier example), on the second day at times 1 and 3 (8 am and 4 pm), and so on for 9 days. At the end of training, birds progressed to the test, on the condition that they had received a reward at each time of day within the last two days. This requirement led to an extension of training for one bird, which had experienced very heavy rain over the last few days of training and did not visit the array at one training period.

Sequential Treatment

In the Sequential Treatment, birds were also presented first with a single rewarded flower in the location of the mini-array rewarded at time 1. Once birds had

fed from this flower, the other three flowers that made up the first mini-array were erected but left empty, and the bird was allowed to feed from this flower 4-6 times. Each session lasted a maximum of 90 minutes. This was longer than the 75 minutes used for the Time of Day treatment as, whilst for the sequential group, the time of day of a session was not important for predicting a reward, it was important for these birds to have experienced each of the preceding rewarded points. After a bird had fed from the rewarded flower 4-6 times, the array was removed, and not less than an hour and a half later, a flower of a different colour, containing 600 µl of 25% sucrose was erected at the site of the second mini-array. Once the bird had fed from this flower, the other three flowers of the second mini-array were erected (but left empty), and the bird again allowed to feed from the rewarded flower 4-6 times. After at least a further hour and a half had elapsed, a different coloured flower was erected at the site of the final mini-array, again containing 600 µl of 25% sucrose, and the same procedure followed.

The day after pre-training, the sequential birds were presented with the full array of 12 flowers three times, with either the first, second or third flower from pre-training rewarded on each occasion, and the reward location corresponding to order of presentation (the flower rewarded first at pretraining was rewarded on the 1st presentation of the day, the flower rewarded second at pretraining was rewarded on the second session of the day, and so forth). All of the other flowers were filled with water.

At each presentation, the birds were allowed to feed from the rewarded flower four to six times as long as time taken to do this was less than an hour and a half. The full array of 12 flowers was presented three times each day (always with the rewarded flower occurring in the sequence 1, 2, 3) with the time of day at which the arrays were presented varied across days to render time of day cues unreliable. Birds were trained

for six days before being tested although progression to the test was dependent upon the bird feeding from each of the three rewarded flowers within the last two days of training. All birds met this criterion.

We recorded the time of each visit to the array and the order in which flowers were visited for all training sessions.

Test

After training, all of the birds were tested to see what form of timing they used to relocate the rewarded flowers. To do this, on the Test Day we presented each male with the full array of 12 flowers just once. For the Sequential birds, this presentation occurred at the median time of the third presentation across training. For the Time of Day birds, the three arrays were presented at the third of the three trained times. For the Test, all flowers were empty. If a bird had learnt to use a sequence to solve this problem, he was expected to visit the flower that was rewarded first in a day, as this was the first time that the bird would have seen the array on this day, despite the test (generally) occurring late in the afternoon, and the first presentation of the array (generally) occurring at some point in the morning during training. If a bird had learnt to use time of day information to solve this task, he was expected to visit the flower rewarded at the 3rd trained time despite the fact that this was never the first rewarded flower of a day.

We recorded both birds' first choices and the number and sequence of flower visits it took for them to visit the correct flower according to their training regime. We took as the null hypothesis for this experiment that birds had learnt which three flowers were rewarded, but nothing about when they were rewarded. This meant a bird had a

one in three chance of visiting the correct flower as his first choice and should on average have visited the correct flower on his second choice.

Results

Training

We constructed a mixed effects model with a binomial error structure for each of the three trained times, modelling whether or not birds chose the correct flower as a function of treatment group (Time of Day or Sequential), training session (1-6), and the interaction between the two, with a random effect of bird. The interaction between treatment and visit was not significant for any of the three times (Time A: model AIC = 77.5, $N = 69$, $Z = 0.53$, $P = 0.596$; Time B: model AIC = 76.9, $N = 67$, Z = 0.32, $P = 0.752$; Time C: model AIC = 82.6, $N= 67$, $Z = 0.99$, $P = 0.324$) . After model simplification, the only significant factors in each model were visit and the model intercept, with birds becoming more likely to visit the correct flower first across training regardless of whether they had been trained to a sequential or time of day rule (Time A: model AIC = 74.4, $N = 69$, intercept $Z = -3.36$, $P < 0.001$, visit $Z = 2.22$, $P = 0.027$. Time B: model AIC = 73.0, $N = 67$, intercept $Z = -3.20$, $P = 0.001$ visit $Z = 1.97$, $P = 0.049$; Time C: model AIC = 79.7, $N = 67$, intercept $Z = -3.29$, $P < 0.001$, visit $Z = 2.37$, $P = 0.018$, see Figure 2).

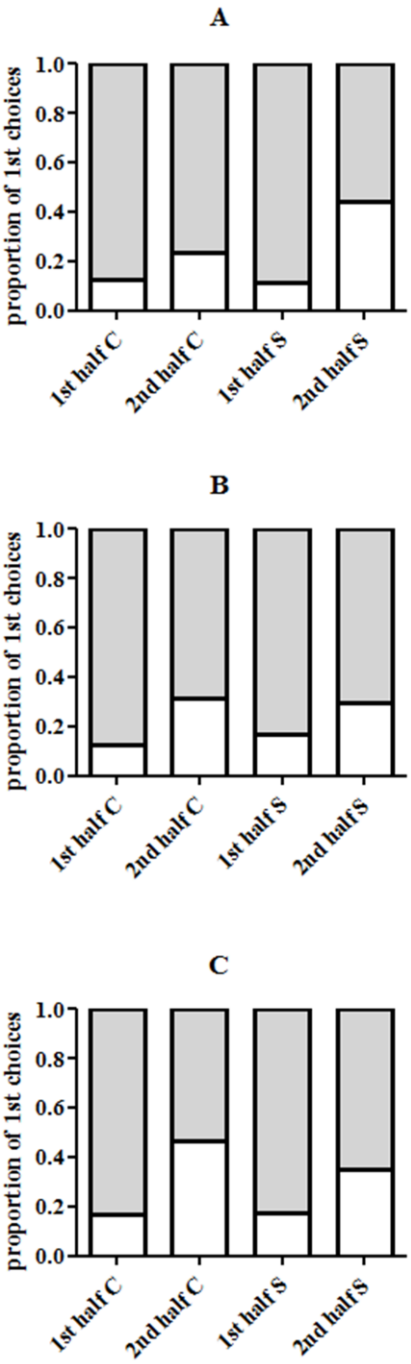

Figure 2. *proportion of correct first choices in the 1st and 2nd half of training for birds in the Sequential and Time of Day group. Clear bars show correct first choices, grey bars show incorrect 1st choices.*

Test performance

At Test, 4 of the 6 birds in the Time of Day Treatment made the correct choice and visited the rewarded flower from Time 3 (Figure 2), a difference between observed and expected proportion of choices of 0.33. However, this was not different to chance (Binomial test, $N = 6$, exact $P = 0.097$). Birds in the Sequential Treatment on the other hand performed very poorly: only 3 of the 6 birds tested visited one of the three flowers that had been rewarded during training as their first choice (Figure 2). Two of these birds chose the flower rewarded at Time 1. The remaining three birds visited flowers that had never been rewarded. As half of the birds made a choice to a flower that was never rewarded, we could not test these birds' performance against chance as we had defined it in the Methods. However, we did compare the proportion of these birds choosing a flower that was one of the rewarded three against chance: they were no better than expected by chance at choosing one of the three rewarded flowers on their first choice (Binomial test, $N = 6$, test proportion 0.25, exact $P = 0.169$; Figure 3).

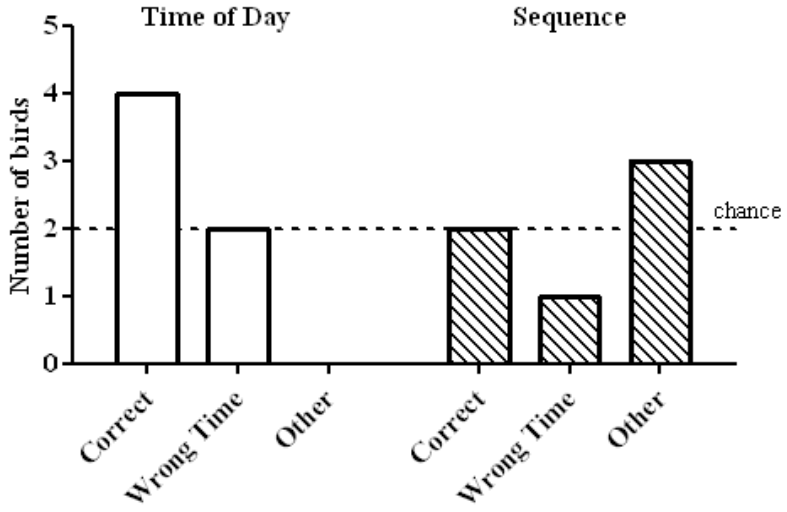

Figure 3. *First choices of birds in the Sequence and Time of Day groups at test, to either the correct flower (time 1 for sequential birds, time 3 for Time of Day birds), flowers rewarded at another time, or flowers that were never rewarded. N=6 for each group.*

We also looked at the number of flower choices the birds made before visiting the correct flower (Figure 4). The Time of Day birds corrected their mistakes very quickly: the two individuals who did not visit the correct flower on their first visit corrected this decision on their second choice. The number of flowers taken to select the correct flower was significantly better than chance for this group (One-sample Wilcoxon signed-rank test: $N = 6$, $Z = -2.00$, P (one-tailed) = 0.023). However, the Sequential birds again did not meet the criterion for chance in this test, visiting many unrewarded flowers, hence their performance could not be tested.

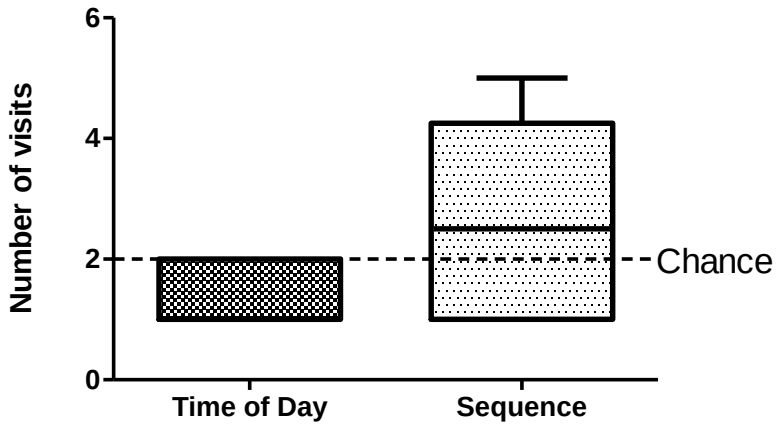

Figure 4*: Number of choices made during the Test by birds in the Time of Day and Sequential groups up to and including visiting the correct flower. Boxes represent the interquartile range and whiskers give the range. The chance line represents birds randomly choosing between the three flowers rewarded during training. N = 6 for each group.*

Discussion

Birds learned either the sequence of rewarded flowers or the appropriate time of day across training, shown by their increasing tendency to visit the rewarded flower first. This supports the conclusions drawn in Chapter 5; that hummingbirds can learn to use both sequential and time of day information to direct their foraging, an important pre-requisite to the conclusion that time of day and sequence may be combined to direct behaviour. However, there was a marked difference between the behaviour of the Sequential and Time of Day group at test. Whilst birds in the Time of Day group performed at above chance levels, birds in the Sequence group did not. Indeed, the birds in the Sequence group did not even meet the criterion we used for chance performance. Whilst the sample sizes used here are small, which means the tests presented here are not very powerful (a potential cause of a lack of significance in Time

of Day birds' first flower choices despite a marked preference for the correct flower), the difference between Sequential and Time of Day birds, with the former choosing unrewarded flowers frequently, and the latter never, is nonetheless striking. This disparity requires further explanation.

One possibility is that, whilst we intended for birds to learn order information in the Sequence treatment, we may not have controlled for another confounding temporal cue, which birds then used preferentially. However, we think this is unlikely. We are not aware of any contextual cue that might have been associated with training (Eacott and Norman, 2004), and we carefully controlled the times of exposure such that time of day could not provide a reliable cue to reward location. This was particularly true at time point 2, which overlapped with both 1 and 3 in terms of time of day, for all birds (see Figure 5). The time interval between the presentations of the arrays was varied across the days of training, and as the start time also varied across days, the birds should not have been able to use interval information to perform correctly the first time they saw an array on a day. On a more general note, there was little consistency between birds in their behaviour at test, and at test birds did not reliably choose flowers from the three rewarded during training. This would seem to imply that, rather than reflecting the use of an alternative time system across birds (which would be expected to produce more consistent results), something about the test itself caused a serious disruption to the birds' ability to choose the appropriate flower.

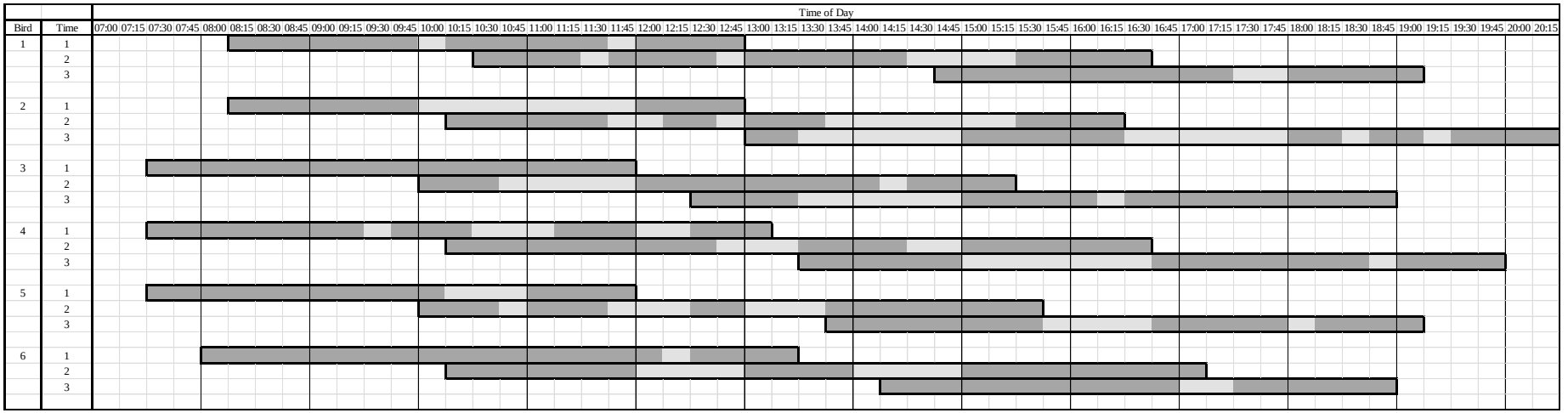

Figure 5*: Diagram of the times of day each bird in the Sequential group experienced times 1, 2 and 3 across training. Solid shading represents times at which birds saw the array, the dotted areas show times at which the arrays were not experienced but which fall within the presentation period for each time.*

At this point, it appears to us that the most likely explanation for the performance of Sequential birds at test is that, whilst they learned the sequence as presented, they also acquired some time of day information. In particular, we suspect that learning of the first sequence element may have been loosely anchored to the morning. This type of combination of different timing cues has been observed in rats *Rattus norvegicus*, which can combine interval and circadian cues (Pizzo and Crystal, 2002), and can combine ordinal information with time of day, such that they can learn that an alternating sequence resets overnight (Carr and Wilkie, 1997). More generally, when animals are taught sequences, there is a generally a signal for the start of the sequence such as black-out (Scarf and Colombo, 2010) or a flash of the house light (Chen et al., 1997). It may be that, whilst time of day information does not provide the only cue to guide hummingbirds' behaviour in this condition, it is providing this sort of starting signal: birds may be recognising the start of the sequence by its occurrence in the morning, then using a sequential strategy to continue foraging. When presented with a test in which they see the array for the first time of day at the time at which they usually see it for the third time, this starting signal is not present and, therefore, the birds do not know where to forage, and appear to approach the array randomly. To examine whether this theory is correct, and whether birds require a clearly identifiable start point to learn a sequence, birds could be tested on a protocol which allowed multiple presentations of a sequence within a day, thus allowing the start time of the sequence to be more thoroughly randomised, and removing any chance that it could be used to guide behaviour.

In comparison with an apparently mixed learning of sequences, the birds' learning of times of day appears to be more straightforward: the Time of Day birds'

performance at choosing the correct flowers both improved over training and their performance at test confirmed that, they had, indeed, learned time of day information. Many, diverse species can use time of day to learn food locations, including bees *Apis mellifera* (Pahl et al., 2007), garden warblers *Sylvia borin* (Biebach et al., 1989, Biebach et al., 1991), rats *Rattus norvegicus* (Mistlberger et al., 1996), and fish *Notemigonus crysoleucas* (Reebs, 1996). We cannot be sure that our birds used a circadian clock here, as opposed to estimating time of day via the position of the sun or timing the interval since sunrise (Biebach et al., 1991), but given that hummingbirds demonstrate daily patterns of foraging in wild conditions (Paton and Carpenter, 1984), as well under laboratory conditions (Gass and Garrison, 1999), and many flower species display daily patterns of nectar availability (Herrera, 1990, Pleasants and Chaplin, 1983, Willson et al., 1979), the use of a circadian clock seems likely.

Hummingbirds are also hypothesised to make use of sequences to guide their foraging, via traplining (Feinsinger and Colwell, 1978), a system in which an animal follows a fixed and repeated route between resources. However, the repeatability of traplines has never been tested empirically in hummingbirds (although Thomson et al. (1997) developed a statistical framework for traplining with bees that may be applicable to hummingbird foraging). The results of our study, by implying that sequences are difficult to learn without the aid of additional temporal information, would suggest that such traplines may be difficult for hummingbirds to acquire. On the other hand, traplines are often posited as an alternative to territoriality for many hummingbird species, as a method of defence by exploitation (Gill, 1988), and it is possible that species that do not defend territories, as do rufous hummingbirds, are better able to learn sequences without time of day information. This kind of variation among species

in memory ability appears to occur in spatial memory (Biegler et al., 2001, Brodbeck, 1994, Hampton and Shettleworth, 1996), and it is possible that a similar effect occurs with temporal memory.

These data provide good evidence that birds can use time of day information to guide their foraging, and some evidence that they can also acquire sequences, albeit with the presence of extra information. Given that sequential learning may be important in hummingbird foraging, it would be productive to increase our understanding of how birds forage in nature. To allow a more exact parallel to traplining to be drawn, a logical next step would be to investigate how birds behave with sequences in which each flower is only rewarded once per sequence. This would also allow birds to complete multiple trials per day, allowing us to establish whether sequences can be acquired in situations where time of day information cannot provide any information about which flower is rewarded. If birds are able to learn sequences in this type of situation, it would suggest that traplining is a cognitively plausible explanation for how non-teritorial birds organise their foraging in the wild. Beyond this, if birds can learn sequences in this sort of situation, it would also be productive to address the forms these sequences take in memory. For example, are they akin to chaining rules, where a sequence is remembered as a series of associations between adjacent elements (e.g. A-B, B-C) as is thought to be the case for pigeons *Columba livia* (although see Scarf and Colombo, 2010), or as a series of ordinal positions (1^{st} = A, 2^{nd} = B, 3^{rd} = C), an arguably more complicated form, found in monkeys *Macaca mulatta* (Orlov et al., 2000, Chen et al., 1997). This second form can more easily be adjusted to incorporate new sequence elements (Terrace et al., 1995), and may therefore be more appropriate to

nectar foragers, as patches may come in and out of reward availability independently of each other.

7: Testing a human model of hummingbird foraging

Introduction

Finding animal models of episodic memory has been the focus of extensive research for the last 14 years. However, as there is still considerable debate as to the similarity between animal models and human memories, progress has not been straightforward (Clayton et al., 2003b, Dwyer and Clayton, 2002, Suddendorf and Busby, 2003a, Suddendorf and Busby, 2003b). While extensive research on episodic-like memory has led to a broad body of support for the idea that animals have a memory system that functions in a way that is similar to episodic memory (Zentall, 2005, Roberts, 2007), the relation between episodic-like memory and human episodic memory remains an area of debate because, whilst animal memory is tested mainly in terms of memory content (the what, where and when of past events; Clayton and Dickinson, 1998, Feeney et al., 2009, Ferkin et al., 2008, Zinkivskay et al., 2009), human memory is generally defined through the experience of the subject (Tulving, 1983, 2002). Although suggestions have been made as to ways in which these discrepancies could be addressed (Suddendorf and Busby, 2003b), the use of language to infer a mental state, key to the definitions of human episodic memory, is likely to render this problem essentially impossible to address in other animals.

On the other hand, it does seem feasible that the experimental paradigms that we use to test animals could, with very minor adjustment, be used to test humans. One of the strengths of the current definition of episodic-like memory (remembering what happened where and when about a unique past event) is that it is very well suited to comparative investigation, having been successfully tested in numerous species including rats *Rattus norvegicus* (Eacott and Norman, 2004, Zhou and Crystal, 2009),

meadow voles *Microtus pennsylvanicus* (Ferkin et al., 2008), chickadees *Poecile atricapillus* (Feeney et al., 2009), and magpies *Pica pica* (Zinkivskay et al., 2009). By moving human research into this framework, and testing performance on similar tasks to those used for testing animals, we may be able to gain a better picture of where the similarities and differences between human and animal performance lie, with respect to learning from and about past events. Indeed, this is an approach that was recently used by Holland and Smulders (2011), to test people on a version of Clayton and Dickenson's pioneering scrub jay experiment (1998).

To this end, we developed a human parallel of the Marshall et al. experiment (2012; Chapter 2). In that experiment, hummingbirds foraged for sucrose in flowers that refilled either 10 or 20 minutes after they had been last emptied. Each flower was either individually distinctive or their colour signalled their refill schedule (i.e. like flowers were coloured alike). The hummingbirds could detect the two refill rates but their learning was not facilitated by flower colour, an outcome that we interpreted to be due to overshadowing. In the current experiment, we presented participants with a computer game, in which they played the part of hummingbirds foraging on flowers that refilled either after five or ten minutes. As for the hummingbirds, each flower was individually distinctive for half the participants, and for half the participants flower colour signalled refill schedule but, like the hummingbirds, the humans were not told the duration of the interval before the flowers were refilled. We attempted to parallel the benefits of selecting a full flower and the costs of selecting an empty one using a point system: participants received ten points every time they selected a full flower, and lost a point every time they selected an unrewarded flower, and at the end of the experiment, their score was used to scale their compensation for participation (one mini

chocolate bar per 100 points above zero). We predicted that, like hummingbirds, people would be able to learn to differentiate between these two rates but that, unlike hummingbirds, the people would pay more attention to colour cues and the colour cues provided by the flowers would potentiate learning the refill rates.

Methods

Participants

The participants were 14 students from the University of St Andrews, recruited via a poster in the Department of Psychology. Prior to the experiment, they were briefed that they were participating in an experiment comparing human and hummingbird learning, and that they would play the part of a hummingbird attempting to deduce the rules according to which a set of artificial flowers 'refilled'. Participants who brought electronic devices (including watches) were asked to leave these in a sealed box for the duration of the experiment. Copies of the information given to participants prior to their participation, and the consent form they signed are presented in Appendix 2.

The experiment was run in a test room within the Department of Psychology, in which the participant was left alone for the duration of the experiment, after the experimenter had clarified that the participant understood what they were expected to do. We filmed the behaviour of the participants, using a stationary camera placed behind them. At the end of the experiment they were fully debriefed about the aims of the experiment and rewarded for their participation with an amount of chocolate that was determined by their score. The computer game used was developed to run on Windows in Delphi using Embarcadero® Delphi® 2010 by Jacqueline MacPherson.

The screens used were a 19 inch IIyama Vision Master 1451, and a 16 inch LG Studioworks 775N. This experiment was approved by the University of St Andrews Ethics committee (Appendix 2).

Experimental Procedure

The participants were alternately assigned to the Matched and Unmatched conditions. In the Matched condition, participants were presented with a screen featuring four flowers, two of which 'refilled' after ten minutes and two after five minutes, where flowers with the same colours had the same refill schedules. In the Unmatched condition, all four flowers were coloured differently. Again, two of them refilled after five minutes and two after ten minutes. At the beginning of the experiment, one flower of the four was rewarded, and the other three became rewarded for the first time at a random time within the first five minutes of the experiment if they were five-minute flowers, and ten minutes if they were ten-minute flowers. This was to prevent all flowers refilling synchronously and to encourage participants to pay attention to each flower individually. To find out whether a flower was full or empty, participants were told that they could click on a flower using the computer mouse. This click would prompt a text box to appear giving the state of the flower. They received ten points for selecting a full flower but lost a point if they selected an empty flower. The score was updated on the screen throughout the experiment. The game ran for 90 minutes, after which participants were given a questionnaire in which they were asked to describe the rule they thought had been used to refill the flowers: at set times, after set intervals, in a fixed order, or randomly. A copy of the questionnaire is given in

Appendix 2. Screenshots of the experiment in the matched and unmatched conditions are shown in Figure 1.

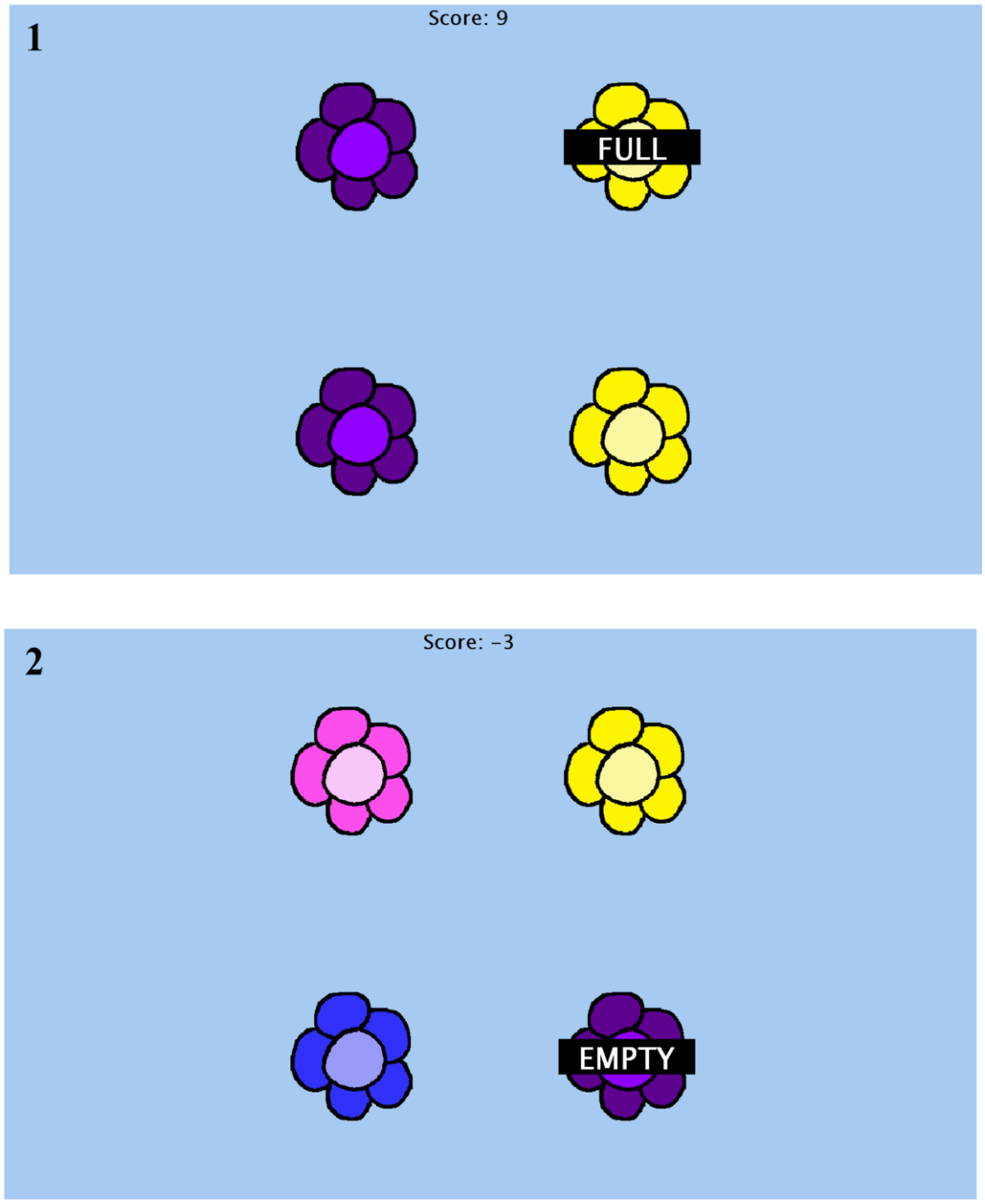

Figure 1*: Screen shots of the experiment as seen by participants in the Matched (1) and Unmatched (2) conditions. The score is displayed at the top of the screen in each case. The participant in the Matched condition has just selected a full flower, the participant in the Unmatched condition an empty flower.*

Results

Scores

We excluded two participants, one from each group, from further analysis, due to their low scores (-3032 in the unmatched group and -1616 in the matched group) and their questionnaire responses, where they reported having been unable to identify any rule governing the flowers' behaviour. The scores of the other participants ranged from -846 to +415. The performance of the Matched group was no better than that of the Unmatched group (One tailed Mann-Whitney U test, $N = 12$, $U = 14.5$, $Z = 0.561$, $r = 0.16$, $P = 0.28$; Figure 2).

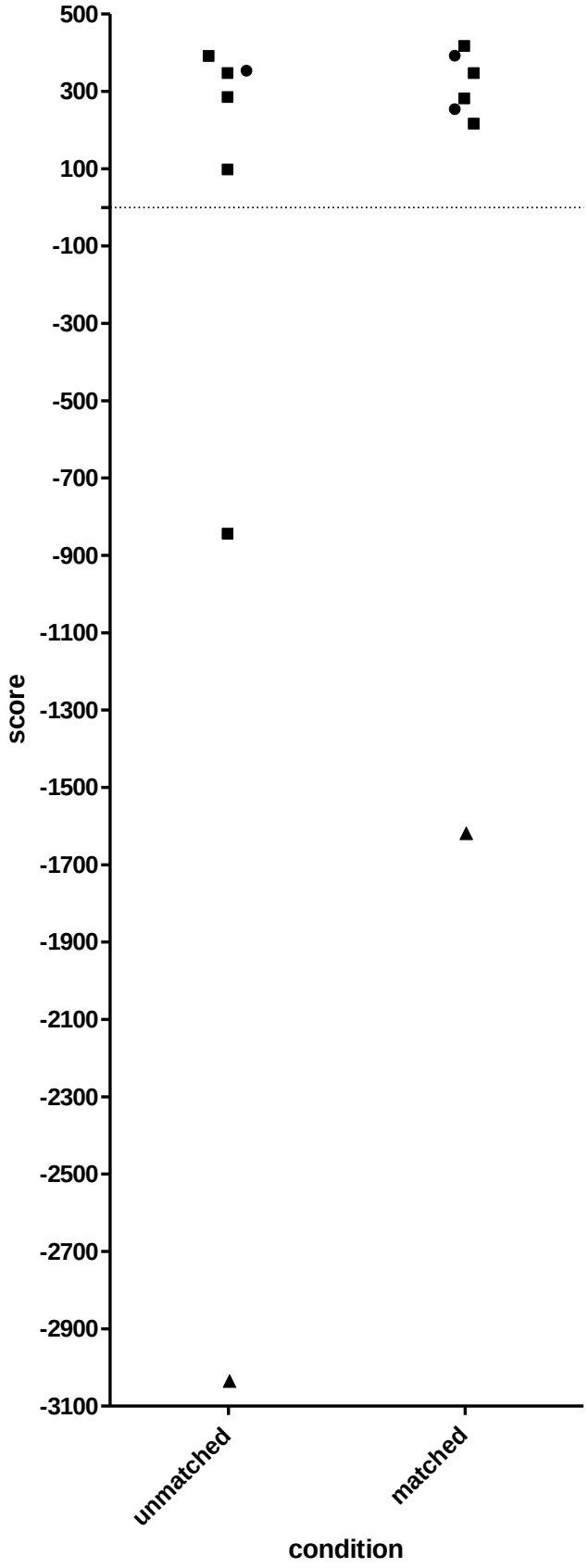

Figure 2: *Scores of participants in the Matched and Unmatched conditions. Circles indicate participants who said flowers refilled in a set order, squares represent participants who identified flowers refilling according to an interval rule and triangles represent participants who thought flowers refilled randomly. The data from these latter two participants (who also scored the lowest) who could not detect any rule governing flowers behaviour were excluded from further analysis.*

Behaviour

We examined several components of the participants' data across the course of the experiment: the post-reinforcement pauses for each flower, the probability of selecting a flower before it had refilled across training and the number of times flowers were selected between each refill. However, as all these measures were resistant to all attempts to model them as a time series, the analyses presented here are based on summary statistics for the second half of the experiment, as a proxy for trained performance. This approach meant that each dataset was tested twice, to establish whether there were differences within treatments between flower types (five or ten minutes) and whether there were differences within a flower type across treatment (Matched or Unmatched). Therefore, in the following analysis, we used a significance level of 0.025.

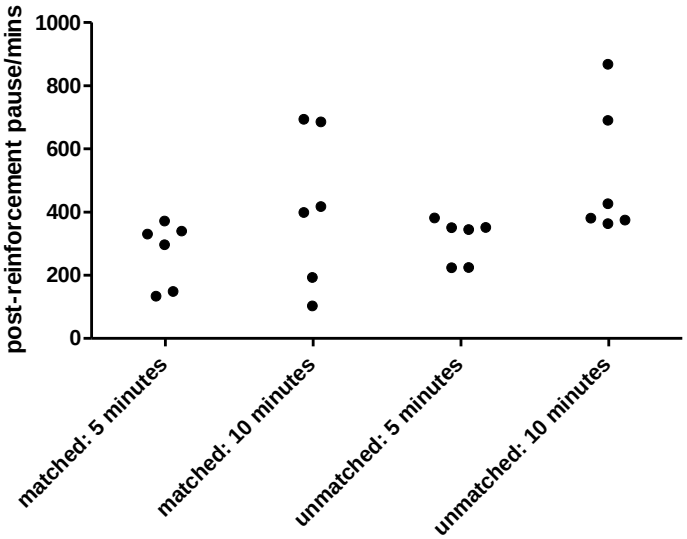

Figure 3. *Median Post-reinforcement pauses for the second half of the experiment for five and ten-minute flowers for participants in the Matched and Unmatched conditions.*

In the Unmatched condition, the participants' median post-reinforcement pauses were significantly shorter for five-minute flowers than for ten-minute flowers (one-tailed Wilcoxon signed rank test, $N = 6$, $Z = 1.99$, $P = 0.023$, $r = 0.57$; Figure 3). This also tended to be the case in the Matched condition (one-tailed Wilcoxon signed rank test, $N = 6$, $Z = 1.78$, $r = 0.51$, $P = 0.038$). For neither the five-minute nor the ten-minute flowers were the median post-reinforcement pauses different between the two conditions (five minute flowers: One-tailed Mann-Whitney U test, $N = 12$, $U = 25$, $Z = 1.21$, $r = 0.35$, $P = 0.155$; ten minute flowers: One-tailed Mann-Whitney U test, $N=12$, $U = 21$, $Z = 0.48$, $r = 0.20$, $P = 0.350$). Participants appeared to choose more empty flowers in the Unmatched condition than in the Matched condition across the whole 90 minutes of training (Figure 4), but this is not reflected in the summary statistics we were able to conduct.

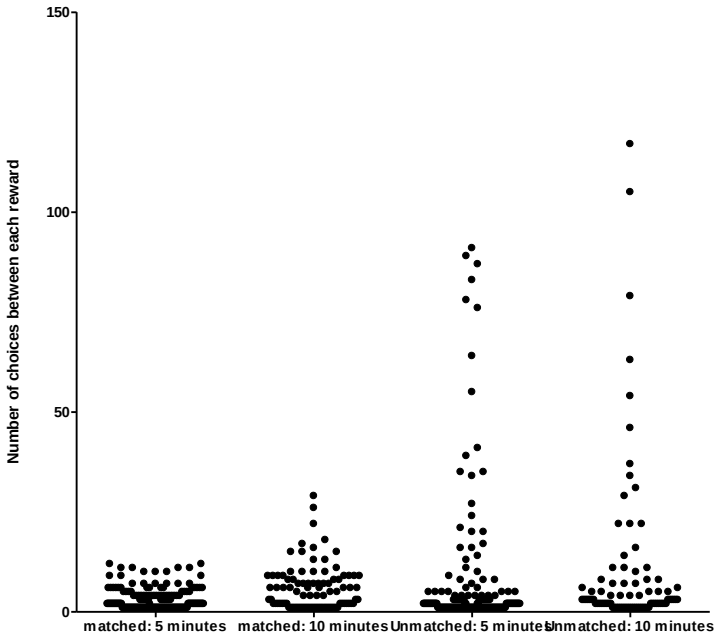

Figure 4. *Number of times a flower was chosen after it was last rewarded across the experiment for all participants. The number of choices minus 1 gives the number of times an empty flower was selected. Lower numbers and greater similarity between five and ten-minute flowers indicates better performance.*

Whilst it was impossible to model participants' data as a time series, to compare learning across groups, we can compare learning performances between the two groups visually. Figure 5 shows the post-reinforcement pauses to five and ten minute flowers by participants in the Matched and Unmatched groups against attempt number (1^{st}, 2^{nd} 3^{rd} etc). As can be seen, participants' performance at ten minute flowers appears to plateau after about 7 attempts for ten minute flowers, and after about 12 attempts for five minute flowers. In real time, this means participants' performance at both flower types plateaued at about the same time (as ten minute flowers take twice as long to refill as five minute flowers, 7 attempts at ten minute flowers will occur at about the same time as 14 attempts for five minute flowers). There is no clear effect of condition (Matched or Unmatched) on learning, with participants in the Matched condition seeming to improve more quickly at the beginning of the training (with regards to both five and ten minute flowers), but tending to plateau at a lower level. The tendency of the participants' PRPs to ten minute flowers to decrease towards the end of training is likely an artefact of the fact that participants whose performance was poor generated more post reinforcement pauses, as flowers were selected as soon as they refilled (at the cost of making many incorrect selections), hence the tails of these lines represent the performance of the worst participants.

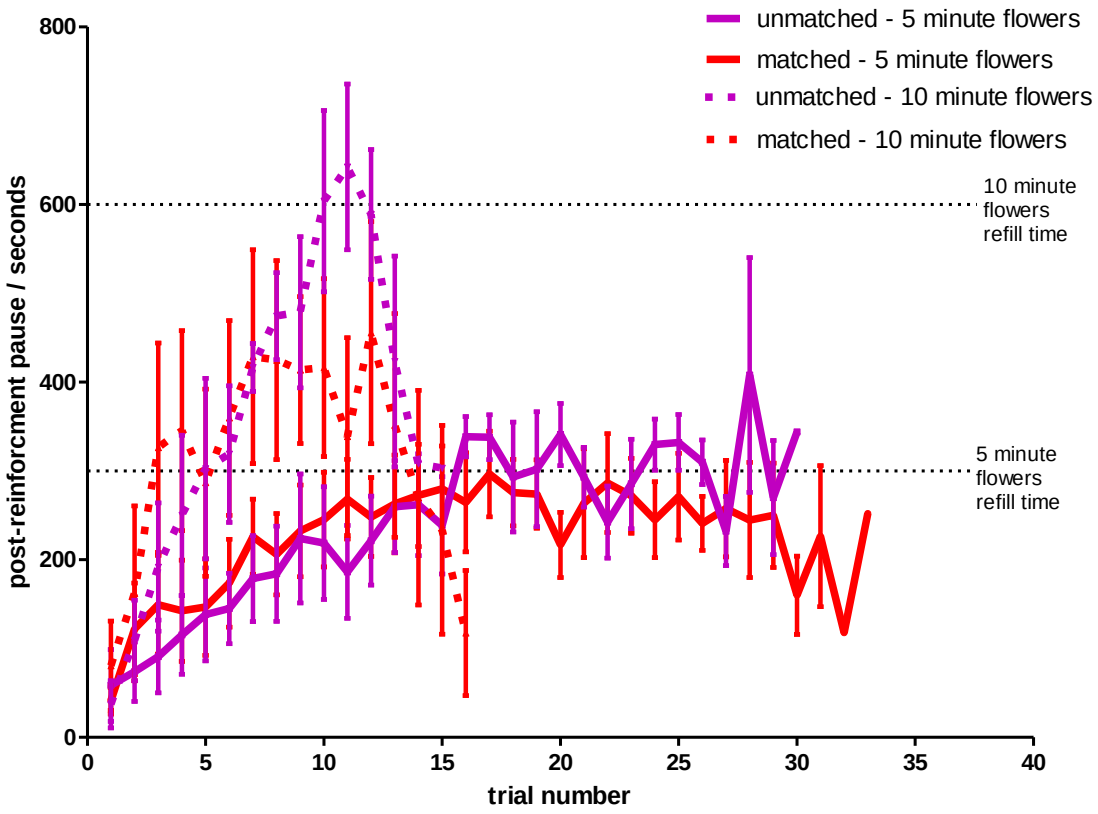

Figure 5*: post-reinforcement pauses for the matched and unmatched condition to five and ten minute flowers, against attempt number. Matched condition given by red lines, unmatched condition by purple lines. Solid lines give five minute flowers, dotted lines indicate ten minute flowers. Error bars: ± S.E.M. N =6 for each group.*

Questionnaire responses

Two participants thought the flowers refilled randomly. They also scored the lowest of all the participants. All of the other participants either thought that flowers refilled in a set order or at set intervals. Both of these strategies were fairly successful. It is also noteworthy that, across participants, there did not seem to be a large difference in how successful order and interval strategies were (see Figure 2).

Discussion

Participants in both groups were able to learn to wait longer before selecting ten minute flowers than five minute flowers after that flower had last been rewarded, significantly so in the Unmatched group, and to a near significant level in the Matched group. It appears, then, that the participants were able to learn to solve a task similar to that posed to hummingbirds in Marshall et al. (2012). It appears that, like the hummingbirds, colour made little difference to the speed with which the participants' learned the refill rates. Whilst Figure 5 suggests participants in the Matched group may have learned more quickly, if anything, participants trained performance in the Unmatched group was slightly better, and the groups did not differ significantly in their final score or in their post-reinforcement pauses to faster or to slower-refilling flowers with effects sizes suggesting, if anything, a small to moderate effect in the opposite direction to that predicted (i.e. participants in the Unmatched group outperforming the Matched group), consistent with Figure 5. However, the types of analysis that we could conduct on the data are comparatively insensitive, especially given the small sample sizes available and the noise we tend to see in post-reinforcement pause data, and hence drawing a conclusion regarding the effects of colour on the basis of this analysis is difficult, a point highlighted by the fact that participants did not quite differentiate between five and ten minute flowers at statistically significant levels, despite a large effect size ($r = 0.51$).

Our ability to ask participants how they solved the task is one point in which this experiment diverges from that of Marshall et al. (2012), and participants' responses to the question of how they organised their behaviour are potentially interesting. Whilst most participants correctly identified that flowers refilled according to elapsed intervals

(four in the Matched group and five in the Unmatched group), other participants used sequential strategies to determine which flower to select (two in the matched group and one in the unmatched group). The difference in strategy does not appear to have made a great difference to participants' performance (Figure 2). If the tendency not to use a sequential strategy is indicative of ease, then possibly, as they are for hummingbirds, sequences may be more difficult for humans to learn than is learning time intervals.

More broadly, this experiment serves as a proof of principle that it is possible to adapt the system used to study hummingbird memory to human participants, and suggests it may be possible to adapt other episodic-like tasks using similar timescales for use with human participants. One question that such an approach may be used to address is what strategy animals use to estimate how long ago an event happened with regard to these sorts of timescales (tens of minutes to hours). In the episodic-like memory literature, there has much debate regarding the nature of the "when" animals use to solve a what, where, when task in which the when is encoded in terms of how long ago an event occurred (Raby and Clayton, 2009, Roberts, 2002, Roberts et al., 2008, Suddendorf and Corballis, 2007) . In particular, some proposed systems for making this sort of judgement are thought to be prospective, such as interval timing (Gibbon, 1991), in that they rely on mental processes that begin at the moment of the first event and are updated through the interval to be timed, such that at the point at which an animal makes a choice, no retrospection (remembering back to the first event) is required. Research on human duration estimation suggests that people possess both prospective systems of this sort (which rely on keeping track of a duration as it elapses), and retrospective systems, in which intervals are not tracked as they elapse, but estimated after their conclusion by remembering the contents of the interval. Of the

two, it is this latter system that appears to have more in common with episodic memory (Zakay and Block, 1997). These two systems are experimentally dissociable: for example, prospective systems rely on working memory and thus when attention is divided participants tend to underestimate the to-be timed duration (effectively they "miss" time passing as they are not paying attention to it). On the other hand, a person using a retrospective system will tend to overestimate durations of intervals that are highly segmented, an effect thought to be caused by using the contents of an interval to estimate its duration (Block and Zakay, 1997, Block et al., 2010). By using models appropriate to both humans and other animals, such as the human/ hummingbird paradigm we have developed here, to investigate the effects of these sort of changes on human and hummingbird timing, we may be able to further elucidate how animals estimate time in tests of episodic-like memory where the intervals defining the "When" aspect of memory are relatively short., whether humans estimate such intervals in the same manner, and whether animal performance more generally is consistent with the use of episodic strategies.

8: General Discussion

In this thesis, I set out to examine how animals respond to resources that change with time in the real world. In particular, I wanted to look at what animals can learn, how this information is learnt, and how far what animals learn and remember resembles human memory, all questions that I addressed using free-living male rufous hummingbirds in the Rocky Mountains of Alberta, Canada. As a result, I think we now have a much better understanding of the types of timing system these birds can use to organise their foraging, and how timing information is learned.

At the beginning of this program of work, we were aware that rufous hummingbirds could learn to return appropriately to flowers that refilled after intervals of ten and twenty minutes (Henderson et al., 2006). We have expanded the duration of this capability, having demonstrated that birds can time intervals of up to forty minutes. We have also shown that these birds can differentiate between refill rates when flowers change state more gradually, a situation that may be more akin to natural foraging conditions than is learning fixed refill schedules. Birds can learn to use time of day to direct their foraging, and it seems that sequences can also be learned. Furthermore, hummingbirds can combine information between these systems, as seen in the data in Chapter 5 where our test results seem to be consistent with birds combining time of day and order information to make decisions about which flower to visit. Hummingbird timing, then, seems to be very flexible, with birds being able to acquire rules across a wide range of modalities quickly and able to combine information between them when appropriate. There is, however, some suggestion that these timing systems are not all equal: in particular, it seems that sequences may be harder to learn than are times of day, or at least less able to direct behaviour without additional information from other

systems, for example, without some form of cue to signal the sequence's start (Chapter 6).

This difficulty in learning sequences is somewhat surprising, given that sequence learning has been found in animals including rats (Kesner et al, 1984), pigeons (Scarf and Colombo, 2010), and monkeys (Chen et al., 1997, Orlov et al., 2000), in some cases with a single presentation (Kesner et al., 1984) and is postulated to be important for hummingbird foraging more generally (Feinsinger and Colwell, 1978, Gill, 1988). It is possible that these birds may be more able to learn sequences in different circumstances, for example if sequences are presented over shorter time scales, or in situations where flowers are fed from consecutively (rather than one at a time) and sequences thus become a fixed route through space, as is generally the case with traplining (Feinsinger and Colwell, 1978, Gill, 1988). If birds are found to use sequences to guide their behaviour, there are many interesting questions to be addressed regarding how these sequences are learned (for example, are they chained or ordinal, and how do they adjust to the inclusion or removal of sequence elements). However, the evidence that we have to date suggests that, compared to time of day information or interval information, sequences are the least useful form of time for these birds.

Not only do we now know that different sorts of timing rules can be learned, we have also found out something about how these rules are learned. In Chapter 2 we found that colour does not seem to be an important aid to this learning, at least when the temporal task in question is based on learning intervals, a result that is consistent with hummingbirds' preferences for cues in other circumstances (Hurly and Healy, 1996, Lyerly et al., 1950, Miller et al., 1985), and which may be an example of overshadowing. Further, when birds learn intervals of this sort, they do so in a way that

is consistent with the behaviour of animals tested over shorter durations in interval timing experiments in the laboratory. Specifically, the timing of the hummingbirds exhibits the scalar variance that is a fundamental property of short interval timing (Lejeune and Wearden, 2006), suggesting that the systems controlling short interval timing in the laboratory may also explain the temporal component of What, Where, When memories, at least over this timescale. We also have learned something of how information is learned in What, Where, When tasks. When birds make errors in a What, Where, When task, it seems these mistakes can be attributed to a failure in remembering one of these aspects, and that some of these mistakes are made more often than others: for example, birds seem to find When the most difficult component to learn while What mistakes are most easily corrected, although, given the fact that birds do not use colour in the interval timing, it is unclear whether the What is flower colour or relative spatial position. This suggests that the What, Where, and When aspects of this task are remembered separately, and birds' correct performance is a result of combining these separate pieces of information. This separation of What, Where and When is also a proof of principle of another of our ideas, which is that testing other aspects of human episodic memory in animal models, namely reconstruction, may lead to insights into the nature of episodic-like memory (Hassabis and Maguire, 2007, Schacter and Addis, 2007). Chapter 7 also works as a proof of principle that it may be useful to make comparisons of human episodic and animal episodic-like memory by adapting animal tests for use with human subjects (Holland and Smulders, 2011).

Future work using experimental paradigms like those I have described in my thesis may allow us to make some progress on resolving the question of how alike episodic and episodic-like memory are to each other. Although the questions of how

memory is experienced as raised by Suddendorf and Bubsy (2003b) are not likely to be addressed in the near future, we are able to ask other relevant questions about the similarities between episodic and episodic-like memory. The most straightforward way of doing this may be to test people using the same systems we use to test other animals (Holland and Smulders, 2011), as we attempted to do in the experiment described in Chapter 7. Whilst humans use of episodic memory in tests used to investigate animal models does not necessarily imply that animals use episodic memory to solve the same task, if humans and animals use different systems to solve the same problem then systematic differences in the ways in which the two groups solve the same task should arise across a variety of situations. On the other hand, if over a variety of situations the performances of animals and healthy human participants remains similar, it might be reasonable to conclude that this similarity results from the use of the same kind of cognitive system. A practical advantage of this comparative approach is that it would allow for the same tests to be used in human and animal test subjects, which is crucial for effective drug development.

In a similar vein, we may also be able to make progress in elucidating the similarities and differences between episodic and episodic-like memory by looking at how memories are structured. Episodic memories do not exist as video-like recordings of past events but rather as associations between components that are remembered separately (Schacter and Addis, 2007, Schacter et al., 1998). Although we discuss episodic-like memory with reference to memories as containing bound information about What, Where and When (Clayton et al., 2003a), the form that this binding takes is not yet explicit: episodic-like memories may be formed like episodic memories or they may more closely resemble the working of a video camera. If episodic-like memories

are structured in the same sort of way as episodic memories, and are thus vulnerable to the same sorts of errors and distortions, we would have stronger grounds for arguing that the two systems are equivalent in form as well as in function. Questions of the structure of memory may be addressed either by behavioural studies or by lesion work. The relationship between memory deficits and neurology is, however, not yet clear. For example, rats with lesions of the postrhinal cortex are impaired at a What, Which task (Norman and Eacott, 2005), which implies that this area of the brain may be responsible for remembering the context in which an object appeared. However, rats with the same lesions perform similarly to intact rats on a What, Where, Which version of the same task (Eacott and Norman, 2004). This result implies that there is some redundancy in memory and that rats may use two distinct processes to solve what appear to be two very similar tasks. If there is, indeed, redundancy (as is often seen in many other behavioural and neural contexts), it may be more appropriate to gather more behavioural data from intact animals, in an effort to understand more comprehensively how What, Where and When are connected to each other in memory, before we attempt to isolate these structures neurologically.

This thesis has shown that there are a variety of timing systems available to the rufous hummingbird, all of which have been used to study episodic-like memory in different species (time of day in rats: Zhou and Crystal 2011; how long ago in scrub jays: Clayton and Dickinson, 1998; order in rats: Fortin et al 2002). In particular, hummingbirds' abilities to keep track of elapsed intervals, and to learn where food can be found on the basis of time of day, are feasible candidates for further study of episodic-like memory. Which of these approaches is more appropriate for making comparisons to human memory, or whether another time system entirely should be used

has been a matter of considerable debate (Roberts, 2002, Crystal, 2009, Easton & Eacott, 2012). As mentioned in the introduction, in the majority of human research, it seems that time tends not to remembered as a separate aspect of memory in of itself but is instead inferred from a memory's content (Friedman, 2004, Friedman, 1993). In some situations, particularly for young children who do not have a grasp of time patterns such as weeks and months, time may also be remembered as an elapsed duration (Friedman, 1991). Given the largely reconstructive nature of time in human episodic memory, the What, Where, Which approach to examining episodic-like memory in animals proposed by Eacott and Norman (2004) is potentially the most similar of all the current models to human memory systems, in that time is inferred from aspects of the memory itself, rather than being remembered as an entity in its own right, and therefore may be most appropriate for animal models wishing to mimic as closely as possible human memory as we have studied it thus far.

However, other forms of time may still have their uses in comparative studies. Sequential approaches (Fortin et al., 2002) and the time of day system used by Zhou and Crystal (2011) also resemble episodic memory in so far as the time of an event is encoded as that event occurs, although evidence for sequential strategies in human memory is somewhat limited (Friedman, 1993). Time judgements based on how long ago differ from sequences or time of day in that how long ago an event occurred is constantly changing and, therefore, is not encoded at the time of the event itself but rather is established at the point of making a memory judgement, a process that could occur via numerous different mechanisms, such as assessment of the strength of a decaying memory trace or by interval timing. However, how long ago judgements are used by humans in some circumstances to date their episodic memories, in particular by

small children (Friedman, 1991), who lack the knowledge of time patterns necessary to reconstruct times of occurrence onto larger timescales in order to date events. This may mean that how long ago is also a common form for the time aspect of episodic memory in animals, which, like small children, are unlikely to have concepts of long scale cyclical time patterns such as weeks and months for reference when reconstructing the times of their memories. This suggests that defining when an event occurred in terms of how long ago it happened may still be a productive avenue for future research in animals.

How long ago has proved not only the most popular way of testing for memory of when an event occurred in episodic-like memory, but also the most contentious. In particular, there has been a great deal of debate regarding how these durations are tracked, with counting days, decaying memory traces, and interval timing systems all being candidate mechanisms (Raby and Clayton, 2009, Roberts et al., 2008, Suddendorf and Corballis, 2007, Roberts, 2002). The results of Chapter 3 in showing that hummingbirds keeping track of elapsed time over relatively long intervals in naturalistic settings do so in a manner consistent with an interval timing system may have some bearing on this argument. If interval timing systems can commonly be used to judge how long ago problems by animals, then how these intervals are tracked becomes very important. Under prospective models such as SET (Gibbon, 1977, 1991), time is estimated by processes which commence at the beginning of the interval and are updated throughout the timed interval, thus require no memory of the starting event to guide behaviour. On the other hand, alternative models such as MTS (Staddon and Higa, 1999), which rely on characteristics of memory for an event, namely memory decay, as a proxy for elapsed time may be more compatible with episodic-like memory.

Using the same systems to test humans as animals, as attempted in Chapter 7, along with what we know about how prospective and retrospective duration estimates are affected by events within to-be-timed intervals (Block and Zakay, 1997, Zakay and Block, 1997, Block et al., 2010) may also help us to determine whether prospective or retrospective systems are used to make these judgements, whether humans and animals differ in this regard, and therefore how appropriate systems using how long ago are for drawing comparisons between episodic and episodic-like memory.

One important caveat to the use of how long ago as a source of time in episodic-like memory studies is that, whilst it can be used to specify when an event occurred in human episodic memory, successful identification of which of two events happened longer ago can be achieved by humans without using episodic memory. In particular, relative familiarity has been proposed as a correlate of how long ago an event occurred that does not require memory of an event itself. Familiarity provides an alternative to episodic memory in humans, which is preserved in amnesiac patients, and which can enable participants to perform successfully in list recognition tasks (Yonelinas, 2002). The importance of this distinction is particularly indicated by Easton et al. (2012), who found that while humans mostly used episodic memory to solve a What, Where, When task, they could still perform better than chance even when they said they were unable to remember an event. This did not occur when participants had to solve a similar What, Where, Which task that was based on the same stimuli. It has been suggested that the participants were able to assess differences in familiarity between stimuli to identify whether it occurred longer ago or more recently. Distinguishing whether responses in What, Where, When tasks are underpinned by memory for an event or solely by the familiarity of a stimulus is therefore an important question for

experimenters to address if we are to use how long ago to assess animals' episodic-like memories. Given this difficulty, it may be that using a What, Where, Which paradigm to further investigate the similarities and differences between episodic and episodic-like memory is more straightforward than using a What, Where, When paradigm.

As well as looking at how birds behave towards flowers that refill discretely, I also investigated birds' behaviour towards flowers that refill gradually over time (Chapter 4). Here, we found that, whilst birds could detect differences in refill rates, and organise their visits accordingly, they did not match revisit rates to refill rates, as we expected them to do if they were foraging optimally. Apparent violations of the predictions of optimality in foraging are not uncommon (Houston et al., 2007), and we suspect this is because our predictions of what is optimal miss some of the demands of the situation, potentially that birds are foraging for information or engaged in defence by exploitation. To definitively demonstrate which of these is the case, however, would require further research, preferably in a laboratory setting where other aspects of the environment can be more easily controlled.

Another way in which the research presented here has bearing on how wild animals deal with the challenges they are posed by their environment is with regards to the potential it demonstrates for studying the ability of animals to remember time in its own right. Here, we have illustrated that rufous hummingbirds are able to learn to use multiple different timing systems in free-living situations. Of these, the system that we know the least about with regards to hummingbirds is sequential learning, a system of particular interest given the prominence of traplining in theories of hummingbird foraging. A useful next step for examining sequence learning would be to test whether birds were able to learn sequences in situations without the start time cue that we

suspect they may have used in the experiment in Chapter 6. One way to do this would be to test animals on the same sequence multiple times in a day. We could then investigate, if birds were able to learn these sequences, whether they were ordinal or chained. In terms of the different timing systems available to birds, we have not begun to address whether birds can learn a "Which" form of time. In addition, whilst we have some information regarding how these time systems might interact with one another, in particular that times of day and sequential rules may be combined to control behaviour, our knowledge of how these systems interact with each other is limited. Future work could therefore look at whether times from other systems are combined together in a similar manner, or whether in some circumstances information from one timing modality might overshadow information from another.

More broadly, the variety of timing systems used by these hummingbirds suggests that hummingbirds may have great potential for comparative research into the relationships between ecology and cognition. The extent to which differences in species' cognitive abilities can be predicted by differences in the cognitive demands placed on them by their environment remains somewhat controversial. The best evidence we have so far that an animal's cognitive abilities reflect the challenges it faces in the world come from comparative studies of spatial cognition. For example, male meadow voles, *Microtus pennsylvanicus*, whose ranges expand in the breeding season to increase contact with females, have better spatial abilities than do conspecific females or males of the closely related prairie vole, *Microtus ochrogaster*, which do not enlarge their range when breeding (Gaulin and Fitzgerald, 1989). Similarly, food storing birds have a larger hippocampus than do nonstoring species (Krebs et al., 1989, Sherry et al., 1989) and longer-lived spatial memories (Biegler et al., 2001). However,

the question of whether an animal's ecology can be used to predict its cognitive abilities is yet to be addressed in other domains, and thus how much of an influence ecology exerts on cognition remains an open question.

The range of timing systems used by wild rufous males suggests that investigating timing could provide another avenue for investigating how animals' cognitive abilities are shaped by the environment they live in and the way they interact with it. The male rufous hummingbirds I tested here are territorial, central-place foragers but this is not the only foraging strategy that hummingbirds follow. In particular, female rufous hummingbirds during the breeding season do not defend feeders and may be either traplining or feeding opportunistically. Different hummingbird species have also been described as trapliners (Feinsinger and Colwell, 1978, Gill, 1988) and rufous males are not always territorial. Investigating how these different foraging modes within and between species and genders influence the readiness with which different timing systems are learned, and what timing systems can be learned, is a question akin to the investigations that have been conducted on spatial memory.

The question of differences in timing abilities across different species also adds another dimension to work conducted with spatial memory, as there is the potential for natural selection to have favoured one system over another, depending on the animal's ecology. In comparative studies of spatial memory, we have tended to look at space as a unitary aspect of the environment, to which an animal needs to pay more or less attention. In the temporal situation examined in the work in this thesis, on the other hand, it may be that different ecologies favour animals that pay attention to a specific aspect of time. For example, traplining may favour sequential learning, whereas central-

place foragers may be comparatively better at interval timing. That selection may have acted differently on these different time keeping systems is supported by the evidence that these timing systems seem to be served by different brain areas: for example, in mammals, sequential learning is located in the hippocampus (Kesner et al., 2002) and circadian timing in the suprachiasmatic nuclei in the anterior hypothalamus (Reppert and Weaver, 2002). There is, therefore, the potential that, if we see between-species differences in preferred timing systems, we may also see differences in the relative size of the relevant brain regions. Additionally, because hummingbird feeding ecologies vary across seasons in individuals of the same gender, studying hummingbird timing could also be used to look at whether experience is important in determining abilities with different modalities of information, and how experience might interact with evolved predispositions to use one form of temporal information over another.

In sum, how animals cope with temporal problems is an area with great scope for addressing important questions of how alike human and animal memories are and how animals' cognitive abilities relate to the questions posed to them by their environments. By broadening the scope of our research efforts from just looking at whether animals can remember What, Where and When, to looking for the characteristics of episodic memory that can be investigated behaviourally in animal systems, and by looking at ways of making human tests of memory more like animal tests, rather than just trying to make animal models match human testing paradigms as closely as possible, there seems to be real potential to make substantial progress in solving a thorny issue in comparative psychology. By moving timing systems out of the laboratory and into the real world, and looking at them in a more unified manner, there is also the potential that we could gain some valuable insight into how real animals cope

with changing environments, and how these cognitive abilities are (or are not) tailored by natural selection to the enable an animal to cope with that changing environment.

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Appendix 1:

The following pdf has been published as “Marshall, R.E.S., Hurly, T.A. & Healy, S.D. (2012)."Do a flower’s features help hummingbirds to learn its contents and refill rate?" Animal Behaviour **83**: 1163-1169.”
DOI: https://doi.org/10.1016/j.anbehav.2012.02.003

Appendix 2:

This appendix comprises the Ethical approval form for Chapter 7, and all the materials seen by participants in that experiment.

University of St Andrews

University Teaching and Research Ethics Committee

3 November 2010

Ethics Reference No: *Please quote this ref on all correspondence*	PS6945
Project Title:	Refilling resources in foraging model
Researchers Name(s):	Rachael Marshall
Supervisor(s):	Dr Sue Healy

Thank you for submitting your application which was considered at the Psychology School Ethics Committee meeting on the 2nd November 2010. The following documents were reviewed:

1. Ethical Application Form 02/11/2010
2. Participant Information Sheet 02/11/2010
3. Consent Form 02/11/2010
4. Debriefing Form 02/11/2010
5. Advertisement 02/11/2010
6. Questionnaire 02/11/2010

The University Teaching and Research Ethics Committee (UTREC) approves this study from an ethical point of view. Please note that where approval is given by a School Ethics Committee that committee is part of UTREC and is delegated to act for UTREC.

Approval is given for three years. Projects, which have not commenced within two years of original approval, must be re-submitted to your School Ethics Committee.

You must inform your School Ethics Committee when the research has been completed. If you are unable to complete your research within the 3 three year validation period, you will be required to write to your School Ethics Committee and to UTREC (where approval was given by UTREC) to request an extension or you will need to re-apply.

Any serious adverse events or significant change which occurs in connection with this study and/or which may alter its ethical consideration, must be reported immediately to the School Ethics Committee, and an Ethical Amendment Form submitted where appropriate.

Approval is given on the understanding that the 'Guidelines for Ethical Research Practice' (http://www.st-andrews.ac.uk/media/UTRECguidelines%20Feb%2008.pdf) are adhered to.

Yours sincerely

Convenor of the School Ethics Committee OR Convener of UTREC

Ccs Dr Sue Healy (Supervisor)
School Ethics Committee

University of St Andrews

Participant Information Sheet

Project Title: Refilling resources in a foraging model

What is the study about?

We invite you to participate in a research project about foraging. The aim of this task is to see how good people are at exploiting refilling resources, similar to those encountered by nectar feeders such as hummingbirds. In this experiment, you play the part of a hummingbird, feeding from flowers to get food. However, instead of sucrose, the flowers are rewarded with chocolate. These flowers will each refill according to a particular rule, and your aim will be to try and match your selections of each flower as closely to these rules as possible. This is a computer based task, which takes place in a test room in the Department of Psychology.

This study is being conducted as part of my, (Rachael Marshall), PhD Thesis in the School of Psychology.

Do I have to take Part?

This information sheet has been written to help you decide if you would like to take part. It is up to you and you alone whether or not to take part. If you do decide to take part you will be free to withdraw at any time without providing a reason.

What would I be required to do?

You will be asked to play a computer game, based on hummingbird foraging. You will see four flowers on a screen, which will fill according to a set of rules to give you a reward. Flowers are either full or empty- never partially full. To select a flower and find out if it is full, click on it with the computer mouse. Every time you click on a flower that has refilled, you will receive 10 points. Each time you click on an empty flower, it will cost you one point. This is to mimic the cost to a wild animal of making an incorrect foraging decision. At the end of the experiment your points will be scaled to an amount of chocolate which you will receive in return for your participation. You will be able to see your score throughout the experiment at the top of the screen.

To make this as like the challenge an animal would face as possible, you are requested to refrain from using any electronic aides or writing material during this experiment. We request that you remove all electronic or mechanical devices (eg mp3 players, mobile phones, watches, calculators, cameras, netbooks or dictophones) from your person and place them in the covered box on the table in front of you. If you do not wish to do so, you are free to withdraw without giving a reason.

This experiment runs for 1 and a half hours, at the end of which there will be a brief questionnaire, asking about what you think the flowers are doing and how you estimated when to select each one. If you become uncomfortable or wish to stop for any reason, you are free to withdraw at any time without giving a reason. If you withdraw, all data collected will be destroyed without being viewed. If there are any questions on the questionnaire that you do not wish to answer, you are free to leave them blank.

During this experiment you will be filmed, in order to see how people behave when doing this sort of task. If you are not happy with this, you may withdraw at any point without giving a reason. If you do this, any footage or data collected prior to your decision will be destroyed and will never be viewed. The camera will be positioned behind you and angled towards the screen.

Will my participation be Anonymous and Confidential?

Only Rachael Marshall and Dr Healy will have access to the data which will be kept strictly confidential, and stored in a coded format. This means that your video footage and information about your performance in the task will be identified with an ID number. This ID number will appear on your consent form, which will be stored separately to your other data in a locked filing cabinet. This is done to allow us to delete your data if you choose to withdraw consent at a later date. Your permission is sought in the Participant Consent form for the data you provide to be used for future scholarly purposes.

Storage and Destruction of Data Collected

The data we collect will be accessible by Rachael Marshall and Dr S Healy. We seek your permission for your data and footage to also be made available to other bona fide reasearchers who may wish to make use of it in the future. If you do not wish for us to do this you may withdraw at any time, or contact either Rachael Marshall or Dr Healy at any time after the completion of the experiment and request your footage or data be destroyed. Your data will be stored on a password protected computer in the psychology department, and archived for future use. Your consent form will be stored separately in a locked filing cabinet.

What will happen to the results of the research study?

The results will be finalised by 2012 and written up as part of my/PhD Thesis. They may also be used to generate a research paper for publication in a journal.

Reward

Whilst completing this task, you will receive points, which will be scaled to give a chocolate reward at the end of the experiment.

Are there any potential risks to taking part?

There are no anticipated risks to taking part, however if participation makes you uncomfortable or you wish to withdraw you are free to do so at any time without giving a reason.

Questions

You will have the opportunity to ask any questions in relation to this project before giving completing a Consent Form.

Consent and Approval

This research proposal has been scrutinised and been granted Ethical Approval through the University ethical approval process.

What should I do if I have concerns about this study?

A full outline of the procedures governed by the University Teaching and Research Ethical Committee is available at: //www.st-andrews.ac.uk/utrec/complaints/

Contact Details

Researcher: *Rachael Marshall*

Contact Details: *rm792@st-andrews.ac.uk*

Supervisor: *Dr Sue Healy*

Contact Details: *susan.healy@st-andrews.ac.uk*

Participant Consent Form

Project Title

Refilling resources in a foraging model

Researcher(s) Name(s)
Rachael Marshall, rm792@st-andrews.ac.uk

Supervisor's Name
Dr S. Healy, sdh11@st-andrews.ac.uk

The University of St Andrews attaches high priority to the ethical conduct of research. We therefore ask you to consider the following points before signing this form. Your signature confirms that you are happy to participate in the study.

Consent

The purpose of this form is to ensure that you are willing to take part in this study and to let you understand what it entails. Signing this form does not commit you to anything you do not wish to do and you are free to withdraw at any stage.

Material gathered during this research will be treated as confidential and securely stored on a password protected computer in the psychology department. Data and video footage will be archived after your participation for potential future use by other bona fide researchers. Please answer each statement concerning the collection and use of the research data.

I have read and understood the information sheet.	☐ Yes	☐ No
I have been given the opportunity to ask questions about the study.	☐ Yes	☐ No
I have had my questions answered satisfactorily.	☐ Yes	☐ No
I understand that I can withdraw from the study at any time without having to give an explanation.	☐ Yes	☐ No
I understand that my data will be kept confidential and that only the researcher(s) and supervisor will have access	☐ Yes	☐ No
I agree to my data (in line with conditions outlined above) being archived and used for further research projects / by other bona fide researchers.	☐ Yes	☐ No
I agree to take part in the study	☐ Yes	☐ No

Part of our research involves taking videos. These recordings will be kept secure and stored separately from consent forms, to which they will be linked by means of a coding system.

Photographs and recorded data can be valuable resources for future studies therefore we ask for your additional consent to maintain data and images for this purpose.

I agree to being videoed	☐ Yes	☐ No
I agree to my video recorded material being published as part of this research	☐ Yes	☐ No
I agree to my video recorded material being used in future studies	☐ Yes	☐ No

Participation in this research is completely voluntary and your consent is required before you can participate in this research. If you decide at a later date that data should be destroyed we will honour your request in writing.

Name in Block Capitals _____

Signature _____

Date _____

University of St Andrews

Participant Debriefing Form

Project Title
Refilling resources in a foraging model

Researcher(s) Name(s)
Rachael Marshall, rm792@st-andrews.ac.uk

Supervisor's Name
Dr Sue Healy, sdh11@st-andrews.ac.uk

Nature of Project

This postgraduate research project was conducted to investigate how well people perform in an analogue of a timing experiment presented to hummingbirds. The flowers you have seen refilled either every five or ten minutes, and we are investigating whether you were able to judge the differences between these two durations without a clock or any other means of timing. Half of participants will have seen four flowers of different colours, whereas the others will have seen two flowers of one colour and two flowers of another, with like coloured flowers having like refill times. Hummingbirds do not perform this kind of task any better when this type of colour matching cue is available, and we wish to see whether people perform any differently. You were filmed to allow us to assess whether you might have been using any behavioural method to assist you in timing the flowers on the screen, such as for example foot tapping or pacing.

Storage of Data

Your data may be used for future scholarly purposes without further contact or permission if you have given permission on the Consent Form. If you no longer wish for your data to be used in this manner you are free to withdraw your consent by contacting either Rachael Marshall or Dr Sue Healy.

What should I do if I have concerns about this study?

A full outline of the procedures governed by the University Teaching and Research Ethical Committee are outlined on their website://www.st-andrews.ac.uk/utrec/complaints/

Contact Details

Researcher: *Rachael Marshall*

Contact Details: *rm792@st-andrews.ac.uk*,

Supervisor: *Dr Sue Healy*

Contact Details: *sdh11@st-andrews.ac.uk*

University of St Andrews

PARTICIPANTS WANTED

Project Title

Refilling resources in a foraging model

This study is being conducted as part of my PhD Thesis in the School of Psychology.

We invite you to participate in a research project looking at how good people are at learning the rules governing refilling resources.

In this experiment, you will play a computer game where the aim is to find rewards in flowers that refill after being emptied according to a particular set of rules. The computer game will last for approximately an hour and a half, after which you will be asked to fill in a short questionnaire on your performance. You will be asked to remove all electronic and mechanical devices from your person during the experiment (these will be placed in a box in the test room), and the experiment will be filmed. All data will be held confidentially by Rachael Marshall and Dr Healy, and will be archived for possible future use by other bona fide researchers. Before agreeing to participate in this research you will be given a Participant Information Sheet that will further detail my research before consenting to participate.

Performance in this task will be scored, and participants will be rewarded with chocolate proportional to their score.

Contact Details

Researcher: *Rachael Marshall*

Contact Details: *rm792@st-andrews.ac.uk*

Supervisor: *Dr Sue Healy*

Contact Details: *Susan.Healy@st-andrews.ac.uk*

Questionnaire

Please help us to analyse your results by answering the following questions. If you do not understand a question or do not wish to answer it for any reason, you may leave it blank.

1) How did you think the flowers were refilling (circle your answer)?

a) They refilled in a set order
b) They refilled at set times
c) They refilled after set intervals
d) They refilled randomly
e) None of the above
f) I don't know

2) If you answered a) in the question above please indicate below the order in which you thought the flowers filled:

A

B

C

D

Flowers refilled in the order……..

3) If you answered c) in question 1, please indicate on the diagram below how long you thought each flower took to refill:

A

B

C

D

Flower A:

Flower B:

Flower C:

Flower D:

Thank you for your help, you have now completed the experiment. You will be given a debriefing form, please email me at rm792@st-andrews.ac.uk if you have any questions.